This is a digital copy of a book that was preserved for generations on library shelves before it was carefully scanned by Google as part of a project to make the world's books discoverable online.

It has survived long enough for the copyright to expire and the book to enter the public domain. A public domain book is one that was never subject to copyright or whose legal copyright term has expired. Whether a book is in the public domain may vary country to country. Public domain books are our gateways to the past, representing a wealth of history, culture and knowledge that's often difficult to discover.

Marks, notations and other marginalia present in the original volume will appear in this file - a reminder of this book's long journey from the publisher to a library and finally to you.

Usage guidelines

Google is proud to partner with libraries to digitize public domain materials and make them widely accessible. Public domain books belong to the public and we are merely their custodians. Nevertheless, this work is expensive, so in order to keep providing this resource, we have taken steps to prevent abuse by commercial parties, including placing technical restrictions on automated querying.

We also ask that you:

+ *Make non-commercial use of the files* We designed Google Book Search for use by individuals, and we request that you use these files for personal, non-commercial purposes.

+ *Refrain from automated querying* Do not send automated queries of any sort to Google's system: If you are conducting research on machine translation, optical character recognition or other areas where access to a large amount of text is helpful, please contact us. We encourage the use of public domain materials for these purposes and may be able to help.

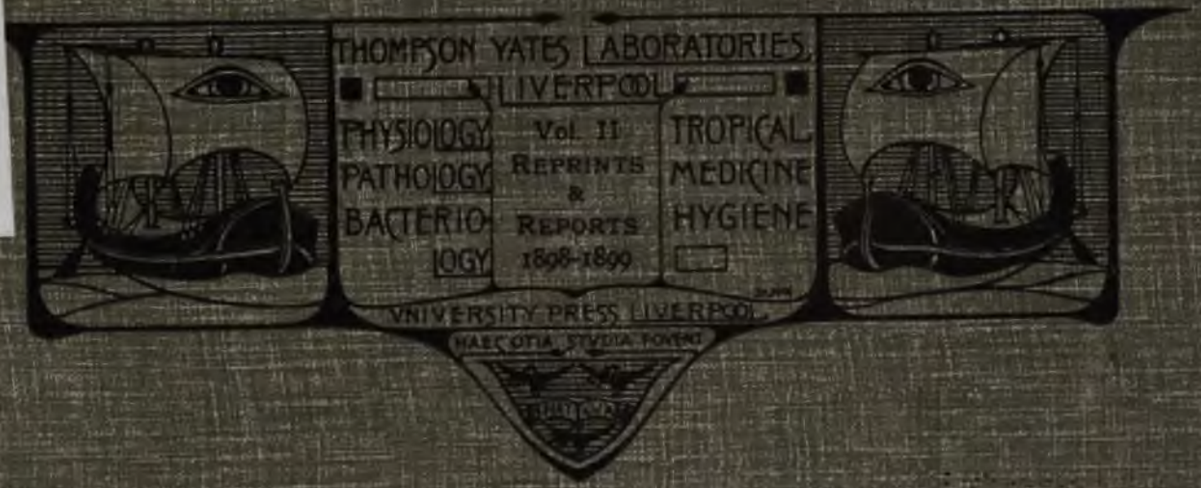
+ *Maintain attribution* The Google "watermark" you see on each file is essential for informing people about this project and helping them find additional materials through Google Book Search. Please do not remove it.

+ *Keep it legal* Whatever your use, remember that you are responsible for ensuring that what you are doing is legal. Do not assume that just because we believe a book is in the public domain for users in the United States, that the work is also in the public domain for users in other countries. Whether a book is still in copyright varies from country to country, and we can't offer guidance on whether any specific use of any specific book is allowed. Please do not assume that a book's appearance in Google Book Search means it can be used in any manner anywhere in the world. Copyright infringement liability can be quite severe.

About Google Book Search

Google's mission is to organize the world's information and to make it universally accessible and useful. Google Book Search helps readers discover the world's books while helping authors and publishers reach new audiences. You can search through the full text of this book on the web at `http://books.google.com/`

LIVERPOOL SCHOOL OF TROPICAL MEDICINE LIBRARY STANDING
2 45 0308 92H



5
3760

H 108
T 47
v.2
1900

LANE
MEDICAL

LIBRARY

THOMPSON YATES LABORATORIES

REPORT

THE

THOMPSON YATES LABORATORIES

REPORT

EDITED BY

RUBERT BOYCE AND C. S. SHERRINGTON

WITH THE CO-OPERATION OF

A. S. GRÜNBAUM AND J. MACDONALD
PHYSIOLOGY AND CHEMICAL PHYSIOLOGY

E. E. LASLETT AND J. EWART
ANIMAL HISTOLOGY

J. HILL ABRAM, W. B. WARRINGTON, C. BALFOUR STEWART, A. S. GRÜNBAUM, C. A. HILL, A. T. MacCONKEY, A. S. GRIFFITH, AND E. E. GLYNN
PATHOLOGY AND BACTERIOLOGY

J. F. RYDER
COMPARATIVE PATHOLOGY

W. B. WARRINGTON
NEUROLOGY

RONALD ROSS, H. E. ANNETT, AND R. FIELDING-OULD
TROPICAL MEDICINE

E. W. HOPE, E. P. MANBY, AND H. R. JONES
HYGIENE

WITH ILLUSTRATIONS AND PLATES

VOLUME II REPRINTS & REPORTS

1898-1899

AT THE UNIVERSITY PRESS OF LIVERPOOL 1900

AT THE UNIVERSITY PRESS OF LIVERPOOL
No. 7. 500 *Copies.* *May*, 1900.

LANE LIBRARY

T47

CONTENTS

Vol. II

BACTERIOLOGY

		PAGE
Blood and the Identification of Bacterial Species	*A. S. Grünbaum*	1
Note on the Specific Action of Normal Human Serum on the *Bacillus coli communis*	*S. R. Christophers*	9
The Bacteriological Diagnosis of Plague	*C. Balfour Stewart*	13
Preliminary Note on some experiments to determine the comparative efficacy of the different constituents of Haffkine's Plague Prophylactic	*C. Balfour Stewart*	17
Experiments to determine the efficacy of the different constituents of Haffkine's Plague Prophylactic	*C. Balfour Stewart*	19
A New Micrococcus with a Note on the Bacteriology of Lymphadenoma	*J. Hill Abram*	23
Mouse Favus	*J. Hill Abram*	27
Tubercle Bacilli in Milk, Butter, and Margarine	*H. E. Annett*	29
The Classification of the Micro-organisms found in Water	*R. Boyce & C. A. Hill*	37
Oysters and Disease *(see Supplement)*	*W. A. Herdman & R. Boyce*	

97416

PAGE

HYGIENE

Parliamentary Powers for the Sanitary Supervision and Control of Ice Cream Manufacture . *E. P. Manby* 41
Meat Inspection and the Abolition of Private Slaughter Houses . . . *E. P. Manby* 45
The Disinfection of the Excreta . *C. A. Hill & J. Hill Abram* 53
Boric Acid and Formalin as Milk Preservatives . *H. E. Annett* 57

MORBID ANATOMY

Carcinoma of the Kidney arising in the Glomeruli . *J. Hill Abram* 69
Two Cases of Lympho-sarcoma and Remarks upon Differential Diagnosis of some general Glandular Enlargements . . . . *J. Hill Abram* 73
The Morbid Anatomy of Dr. Bradshaw's Case of Myelopathic Albumosuria *T. R. Bradshaw & W. B. Warrington* 79

III—Reports

PHYSIOLOGY

Experimental Physiology 91
Histology 91
Chemical Physiology 91
Physical Physiology 92

PATHOLOGY, BACTERIOLOGY, TROPICAL MEDICINE, AND HYGIENE

School of Pathology 93
Municipal Bacteriology 93
Bacteriological Analysis of Food 97
Bacteriological Analysis of Water 113
Pathological Diagnosis Society 119

YALE LIBRARY

PAGE

Museum 123

Comparative Pathology 125

Holt, Alexander, and Colonial Fellowships . . . 127

Preliminary Report on Ophthalmia 129

Tropical Medicine 135

Library 145

SUPPLEMENT

Oysters and Disease . . . *W. A. Herdman & Rubert Boyce*

Report of the Malarial Expedition to West Africa

Ronald Ross, H. E. Annett, E. E. Austen, & R. Fielding-Ould

BLOOD AND THE IDENTIFICATION OF BACTERIAL SPECIES*

By ALBERT S. GRÜNBAUM, M.A., M.D., M.R.C.P.

INTRODUCTORY NOTE

There seems to be some danger of GRÜNBAUM's name disappearing from the literature and from the text-books in describing serum diagnosis, in spite of the custom that when a discovery is made simultaneously and independently by two observers, both names are associated with it; so that we have asked him to reprint his paper in 'Science Progress' on this subject.

We are in a position to know that GRÜNBAUM made his first observation three months before WIDAL's first publication, and the latter has refused, when asked to do so, to say when he made his first serum diagnosis. In any case, in his first papers GRÜNBAUM described certain facts: *e.g.*, (I) The action of human serum on bacilli other than the B. Typhosus. (II) Action of serum from other than typhoid patients. (III) Variability of strength of serum in typhoid patients from day to day. (IV) Necessity for accurate dilution and a time-limit; these have formed the subject of more detailed observation by WIDAL and others without any reference or acknowledgement.

R. BOYCE.

C. S. SHERRINGTON.

Die Unterzeichneten bestätigen gerne, dass Herr Dr. A. S. GRÜNBAUM zu Anfang des Jahres, 1896, an dem Krankenmaterial der I medicinischen Klinik in Wien systematische Versuche über die Agglutination von Typhus und Cholera-bacterien durch das Blutserum von Kranken und Gesunden angestellt hat, und dass er im März, 1896, also lange vor der diesbezüglichen Publication des Herrn WIDAL, an zwei Typhusfällen der Klinik die Beobachtung gemacht hat, dass ihr Blutserum in bedeutender Verdünnung Typhusbacillen agglutinire.

Herr GRÜNBAUM war sich der diagnostischen Verwerthbarkeit dieses Befundes vollkommen bewusst, und er zögerte mit der Publication derselben nur mit Rücksicht auf die Nothwendigkeit die Verlässlichkeit der Reaction an einer grösseren Anzahl von Typhusfällen zu erhärten.

Herr Dr. A. S. GRÜNBAUM hat demnach die Agglutination-reaction des Typhus-Serum selbst-ständig entdeckt und es ist Sache der Gerechtigkeit dies anzuerkennen.

Prof. Dr. NOTHNAGEL.
Docent Dr. MANNABERG.

Wien, am 8. März, 1900.

[TRANSLATION]

The undersigned confirm with pleasure that Dr. A. S. GRÜNBAUM made systematic experiments in the beginning of the year 1896, from the material in No. I. Clinical Ward, in Vienna, on the agglutination of Typhoid and Cholera Bacilli by the Serum of both Sick and Healthy people, and in March, 1896, that is, long before M. WIDAL's publication on this subject, he had observed in two cases of Enteric Fever in the ward that their Serum in considerable dilution agglutinated Typhoid Bacilli.

Dr. GRÜNBAUM was well aware of the diagnostic utility of this observation, and he delayed publishing it only because of the necessity of confirming the reliability of the reaction on a larger number of cases.

Dr. GRÜNBAUM, therefore, discovered the agglutination reaction of Typhoid Serum independently, and to acknowledge this is a matter of justice.

Prof. Dr. NOTHNAGEL.
Docent Dr. MANNABERG.

Vienna, 8th March, 1900.

* From 'Science Progress,' vol. i, No. 5, 1897.

The identification of any particular bacillus may be either a very easy or a very difficult matter. The detection of tubercle bacilli in sputum can be pretty safely entrusted to any conscientious medical student who has done his course of practical pathology: the determination of typhoid bacilli in the excreta of a patient may require all the resources of an experienced bacteriologist. During the last two years, however, the development and proper understanding of previous isolated or misinterpreted observations has given us a new and generally trustworthy method of recognizing many species of micro-organisms. And more than this, by a simple reversal of the procedure it has been possible to supply a new means and aid to clinical diagnosis—another addition to practical medicine derived from experimental science.

So far back as 1889, CHARRIN and ROGER (3) demonstrated that the growth of *B. pyocyaneus* (and of other bacteria) differed when made in the serum of animals immunized against *B. pyocyaneus* and when made in the serum of normal animals. The differences consisted in (i) serum remaining more, transparent, (ii) formation of granular agglomerations, and (iii) microscopically, the formation of chains of altered bacteria, in the former mode of cultivation.

In 1891, METSCHNIKOFF, whilst investigating *Vibrio Metschnikovi*, saw the same phenomena, and noted them as facts requiring further investigation (10). But he made no progress in this direction, being discouraged by failure to obtain the reactions with the bacillus of hog cholera.

In 1893, ISSAEFF made similar observations with PNEUMOCOCCI, and later in conjunction with IVANOFF, with *Vibrio Ivanovi*. They attributed the effects obtained to the bactericidal and growth-hindering substances in the serum (8).

In 1895, WASHBOURN saw similar appearances, and remarked that the mode of growth in the serum seemed to give a good indication of its protective power (13).

All these observers appear to have worked with undiluted serum only, and that too acting for a considerable time. Although they examined the deposit with the microscope, they do not appear to have investigated, microscopically or otherwise, either the immediate effect of the serum on the bacilli, or the effect of diluting the serum.

In 1894, R. PFEIFFER published what is now known as PFEIFFER'S reaction (11), but it was BORDET, who, in 1895, trying to simplify PFEIFFER'S reaction, first described any observations in which the above-mentioned neglected aspects of the question were considered. He saw the agglomeration and loss of movement, but failed to attribute any specific importance to the reaction (2*a*).

It was therefore left to DURHAM and GRUBER, who had already been more than six months at work on the subject when BORDET'S investigations were published, to show how valuable and characteristic this reaction could be made, and to establish its general applicability (4*a*, 6*a*).

In March, 1896, I applied the reaction to the diagnosis or enteric fever in man, but scarcity of material and other delays prevented any paper on the subject appearing before September (7*a*). Meanwhile, WIDAL, profiting by a hint thrown out by GRUBER at the

Wiesbaden Congress (6c) in April, and having more cases at his disposal, was able to publish a communication on serum diagnosis early in July* (14*a*).

PFEIFFER's reaction is observed by injecting a mixture of cholera vibrios, together with a small quantity of serum, from an animal immunized against cholera into the peritoneal cavity of a normal animal. By withdrawing drops of fluid from the abdomen at different intervals it can be seen that the micro-organisms first lose their motility, then become transformed into spherules, and finally disappear. The rapidity and completeness of the reaction depends on various factors (*e.g.*, potency of serum, extent of dilution, etc.). It is not so easily performed with typhoid bacilli.

PFEIFFER thought vital action essential, but BORDET found that the same phenomena could be observed outside the body if the temperature were kept at 37° C. He generally performed the reaction with a mixture of bacilli, 'immune,'† and normal serum; but he recognized that it could occur well with only 'immune' serum, and also, although generally more feebly, with only normal serum from various sources. BORDET laid chief stress on the granular degeneration of the micro-organisms rather than on the agglomeration into groups.

DURHAM and GRUBER examined the phenomena much more thoroughly, and extended it to several kinds of micro-organisms. They found that the addition of a small quantity of 'immune' serum to an emulsion of the corresponding bacillus in a test tube caused a precipitation of the bacilli in the form of fine granules, the supernatant liquid becoming clear. In fact, the reaction resembled an ordinary chemical reaction occurring slowly. A control tube containing only bacilli would remain uniformly turbid. The rapidity and completeness of the reaction were found to depend on the same causes as in PFEIFFER's reaction. If the same experiment is performed with smaller quantities, so that the process can be observed microscopically, under suitable conditions the following stages are seen. The bacilli, at first isolated and moving rapidly about and across the field, gradually slow down and begin to hang on one to another; groups of three or four are thus formed which then adhere to other groups, until, if the reaction be complete, all the micro-organisms are collected into large clumps, with total loss of movement. Should the serum be very potent and not sufficiently diluted, agglomeration and loss of movement may occur instantly, so that by the time the slide is brought under the microscope the reaction is already completed. On the other hand, with a weak or much diluted serum, the phenomena may remain permanently incomplete, so that several isolated micro-organisms are still seen, and even some of those in the clumps may retain their motility. So delicate, however, is the reaction that serum from a very highly immunized animal will produce an effect on its corresponding bacillus even when diluted 500,000 times. The action is to a considerable degree specific (or, as DURHAM prefers to term it, special), for a 'cholera' serum will not act on typhoid bacilli, and 'typhoid' serum will not act on coli bacilli. On the other hand, 'cholera' serum will affect to some extent certain vibrios which are not cholera, and 'typhoid' serum will agglomerate

* Widal has persistently ignored the dates given in my articles, and consequently represents me as having merely confirmed his results. As a matter of fact, so far as I am concerned, the invention of serum diagnosis was made independently, possibly earlier, and arrived at from an opposite point of view.

† For the sake of brevity, 'immune,' 'typhoid,' 'cholera,' etc., serum will be used for the serum of an immunized animal, for the serum of an animal immunized against cholera, typhoid, etc., respectively.

B. enteriditis. But there is generally some difference, and any given serum acts best, viz., in greater dilution, on its own kind of bacillus, so that it can be used for its identification without causing confusion.

The extent to which any serum can be diluted and yet show an effect depends in part on the virulence of the culture employed; the more attenuated the culture, the weaker the serum solution necessary to produce an effect. It is to this grouping power that DURHAM gave the name 'clumping action' of the serum, and the hypothetical body producing it was termed by GRUBER 'agglutinin.'

It is obvious that the reaction can be applied to the identification of various kinds of bacilli if we possess specimens of the corresponding 'immune' sera; and perhaps, as will be indicated later, it may be of use in the discovery of new forms. In spite, therefore, of the sources of error just mentioned, a 'typhoid' or 'cholera' serum can prove of great use in determining whether doubtful organisms belong to these species. It is also clear that the reverse will be true: that by means of a known bacillus we can identify an unknown serum, and can with comparative ease ascertain whether an animal is at all, or to what extent, protected.

That the 'agglutinin' is distinct from the bactericidal and paralyzing substances in 'immune' or other serum appears from the following considerations. An 'immune' serum exhibiting strong agglutinative power need not be appreciably more bactericidal than normal serum. A normal serum may exhibit agglutinative without paralyzing action (7*a*). Agglutinins are not produced in the serum of animals immunized against certain micro-organisms, *e.g.*, *B. diphtheriæ* and certain micrococci.

Motility of the organism is not, as was at first supposed, essential for agglomeration. Not only can agglutination occur using dead, motionless bacilli instead of active, living ones (2*b*), but it may be seen also with some non-motile bacteria and cocci. With some cocci I have seen a phenomenon resembling the loss of movement of motile bacteria. Before the addition of the serum the cocci usually exhibit a Brownian movement, which is much restrained or even entirely inhibited by the action of suitable serum (7*b*).

GRUBER explains the phenomenon of agglomeration by the supposition that the agglutinin causes the enclosing membranes of the bacilli to swell out and become sticky, and that they consequently adhere to each other when they come in contact. He also put forward the idea that the effect of the special agglutinin is thus to facilitate the action of the general bactericidal substances common to all sera; and his theory of immunity is based on these two fundamental ideas.

The theory has not been unanimously accepted. PFEIFFER considers each serum to have a strictly specific action. SALIMBENI does not believe agglutination to occur inside the body (12). Nobody has been able, as yet, to demonstrate a swelling of the capsule. Be all this as it may, the observed facts, without any theory, form a valuable addition to bacterial knowledge.

The 'active' immunization of animals is usually attained by the injection, either hypodermically or into the peritoneal cavity, of dead or attenuated cultures of the micro-organisms. The destruction of the culture used may be effected by the use of either heat or disinfectants, but

too great an application of either diminishes or destroys the immunizing power of the injection (14*b*). The date on which the reaction first appears after injection is rather variable, and depends on two or three factors, although chiefly on the strength of culture. Thus a Guinea-pig, which I injected with living cholera culture on 18th April, 1896, showed the reaction well on the 21st; whilst one injected with dead cholera culture on 16th March, 1896, did not exhibit marked agglutinative action till 23rd March. It is not even necessary to inject the bodies of the bacteria: filtered cultures give similar but weaker or more delayed results (14*b*, 9). By the simultaneous injection of two kinds of micro-organisms agglutinins for both can be produced in the same serum (14*b*).

'Passive' immunization is generally produced by the injection of serum from an actively immunized animal. In such a case the agglutinative property shows itself much sooner. Thus, in a Guinea-pig which I injected at 3 p.m. on 9th March, 1896, agglutinin could be detected in the blood already at 3.30 p.m., and had attained its maximum strength about 6.50 p.m., remaining at the same strength for about one week afterwards. An actively immunized Guinea-pig generally possesses agglutinative serum for a considerably longer period.

The nature of the agglutinating substance has not yet been distinctly ascertained. Filtration of the serum through a porcelain filter diminishes its power. Precipitation by neutral salts of the globulin of the serum or the fibrinogen of the plasma causes loss, or sometimes only diminution, of agglutinative power in the filtered fluid. Similarly serum is generally only two-thirds as powerful as plasma, so that the agglutinin seems to be in some way bound up with the proteids of the blood. Leucocytes, when shed, do not apparently give off any agglutinin. Temperature affects agglutinin in proportion to its height and the time it is allowed to act. Ten minutes at 75° C. destroys the substance, and an hour at 57° C. causes a diminution of effect. Merely heating to 60° C. does not deprive serum of its power (1, 14*b*).

What has been said hitherto relates nearly entirely to animals, and of these, to Guinea-pigs. We shall now consider the question mainly in relation to Man.

The serum of normal Guinea-pigs rarely exhibits agglutinative power, but that of some other animals, particularly of the horse, sometimes show it in a marked but varying degree. So, too, does the serum of Man. The blood of many individuals possesses a pronounced agglomerating action on several forms of micro-organisms; but the quantity of each agglutinin is small, not showing itself when the serum is diluted more than eight times (7*a*). Thus normal human serum presents some striking differences from that of immunized Guinea-pigs, principally in its multiple action. We shall consider later whether this is due to the presence of a common agglutinin or small quantities of each special agglutinin.

When normal serum has any agglomerating action it is often nearly equal on at least three kinds of bacteria—*B. typhosus*, the cholera vibro, and *B. coli communis*. In one diseased condition marked by the evident presence of abnormal substances in the serum, namely, jaundice, the agglutinating action is nearly always present; and if the serum be not diluted, it is as rapid and complete as with the serum of an immunized animal. Neither normal nor icteric serum, however, possesses the power of inhibiting movement. For this reason it seems probable that

during the process of immunizing there are formed in the specific sera two substances, one to cause the agglomeration and another to inhibit movement, for this latter quality is nearly as characteristic of such sera as the former.

If this agglutinative property carry with it a proportionate amount of immunity, then a considerable number of individuals are slightly immunized against typhoid, against cholera, and probably also against other diseases.

It cannot yet be considered settled whether this property is inherited or acquired. Experiments on animals seem to show possibility of inheritance (14*b*), but if in these experiments the inoculations were made into the peritoneal cavity there is an obvious source of error. In the few human cases which I have been able to examine, the blood of the child was usually inactive, although that of the mother was generally, sometimes even powerfully, active. In one instance where the mother had had enterica five years previously, the child's serum exhibited but very feeble agglutinative power. In one case, where the child was born during an attack of enterica in the mother, the serum was very active, but in another it was not so ; possibly in the first case the child was also passing through an attack. If the specific agglutinins are inherited they should be found fairly frequently in the sera of new-born children, especially abroad, where a larger percentage of the population have had either enterica or cholera. According to my own observations, in Austria, at any rate, a greater percentage of adult than infant sera possesses this property, tending to show that it is an acquired quality. It may be that a more extended series of observations would reverse these figures. Anyway, in light of these differences of hæmic properties, statistics on the relative immunity (if it exists) of the children of 'typhoid' parents would be of interest.

It was natural to suppose as the result of experiments on animals, that typhoid (or cholera) patients, who were thus passing through a natural immunizing process, would produce agglutinins in their blood. Observation showed this supposition to be correct, and vaccination experiments on Man have confirmed it. In the latter case agglutinins have been observed in the blood already on the second day after injection (15*b*). When the infection of typhoid takes place in the natural way, owing to the smallness of the initial dose, the agglutinin is not generally found in the serum until two or three days after the nominal commencement of illness, viz., about sixteen days after infection. This may be taken to be about the time when the contest between organism and micro-organism seriously begins. In cholera, with its much shorter incubation period and rapid development, we should expect to, and actually do, find the reaction earlier.

If the normal agglutinin in the blood is of a non-specific character, then an attack of enterica produces an entirely new substance and does not add to what is already present, because the effect of the serum on cholera vibrios and coli bacilli remains the same, only that on typhoid bacilli is increased.

The rate of agglutinin production varies in different cases as might be expected, and there is also a remarkable variation from day to day in the agglutinative power of the serum. These variations can hardly be entirely due to experimental errors. In immunizing animals I have often noticed that the agglutinative power seemed to increase irregularly. Moreover, it is known that

the agglutinin is used up during its action on the bacilli, this being one reason for always employing a constant quantity of culture. Consequently, it appears possible that a rapid increase in the number of micro-organisms, which may easily occur if they light on some fresh suitable soil, would use up the available agglutinin and reduce the amount temporarily below what it had been the day before, and may reduce it below the level of normal blood. And it explains why the most severe cases of typhoid may have comparatively little agglutinin in their blood.

By the aid, therefore, of a known culture of typhoid bacilli, and by suitable dilution of the serum to be tested to avoid any error due to the presence of normal agglutinin, it is possible to diagnose enterica by the agglutinin contained in the patient's blood. It is obvious that the principles of serum diagnosis apply to other diseases as well as enterica. The method has indeed been used for the diagnosis of cholera, Malta fever, and glanders. In diphtheria and tubercle it has not yet been successful. It might very well be applied also to the diagnosis of pneumonia, erysipelas, appendicitis, and influenza, if required, since it is not necessary that the specific organism should be motile. From a case of scarlet fever I isolated a coccus which agglutinates with scarlet fever serum (*7b*).

One general precaution must be mentioned regarding the serum diagnosis of any disease. Although absurdly obvious, neglect of it has already led into error, namely, that the test is not reliable if injections of 'antitoxic' serum have been made and agglutinins thus artificially introduced.

Not only may the agglutinin reaction be applied to the diagnosis of disease already present, but is also likely to be of use in the discovery of specific micro-organisms as yet unknown, *e.g.*, small-pox, typhus, measles, etc. For this purpose bacteria must be isolated from the various tissues, and their reaction with the serum of patients convalescent from the disease in question ascertained. Naturally, any positive result must be controlled with other sera, and by suitable dilution of the specific serum.

There is one source of fallacy against which it is necessary to guard. Some infectious diseases, *e.g.*, scarlet fever, and small-pox in its later stages, are nearly certainly cases of mixed infection, more particularly with the organisms of sepsis. Under such conditions more than micro-organism might give a reaction with the serum, and other criteria would have to decide which is the specific one. Partly for this reason I do not consider the coccus above-mentioned to be the specific organism of scarlet fever.

Without DURHAM and GRUBER's exact and complete investigation and extension of the older isolated observations, a valuable addition to both practical bacteriology and to practical medicine would have been, if nothing else, for some time delayed. The application of the serum test to disease in Man is but the natural outcome of their work. Being now part of routine diagnosis it is past the stage when the name of any individual should be attached to it, but in the history of this new departure in diagnosis their names have the first claim to be mentioned.

October, 1897.

ADDENDUM

An enormous literature has grown around the subject of serum diagnosis. The general tendency is to confirm its utility. From my own experience I am inclined to think, contrary to the usual teaching, that greater accuracy is attained by diluting the serum to at least 1 in 30, and regarding the formation of quite small groups as indicating a positive result, rather than by diluting only 1 in 10 and requiring large groups, clearing of the field, and loss of motility. The latter sign especially I regard as inessential, provided agglutination is present.

My opinion that severe cases might often give feeble results has been confirmed, not only for typhoid but also for other diseases, so that I feel inclined to regard the tentative explanation given in the text as correct.

An important development of the work on agglutination has been its extension to red blood corpuscles, spermatozoa, &c. They can be agglutinated by the serum of different patients or animals (BORDET, LANDSTEINER, DUNGERN, SHATTOCK, &c.). There may be some future practical diagnostic value in these experiments. It is interesting to recall, in this connection, the physical experiment of SIR L. BRUNTON with matches or discs of cork floating in a slightly oily liquid. The addition of acid causes them all to separate; making the solution alkaline causes agglutination.

March, 1900.

BIBLIOGRAPHY

1 ACHARD ET BENSAUDE. Compt. Rend., vol. 123, p. 503.

2 BORDET. (*a*) Ann. de l'Inst. Past., p. 462. 1895. (*b*) *Ibid.*, p. 193. 1896.

3 CHARRIN ET ROGER. Compt. Rend., vol. 109, p. 710.

4 DURHAM. (*a*) Proc. Roy. Soc., vol. 59, p. 224. (*b*) Journal of Pathology, vol. 4, p. 13.

5 FOERSTER. Ztschr. f. Hygiene, vol. 24, p. 500 (gives several references).

6 GRUBER. (*a*) Münch. Med. Wochschr., p. 206. 1896. (*b*) Wiener Klin. Wochschr., p. 183. 1896. (*c*) Verh. d. Congresses f. Inn. Medic., p. 207. 1896.

7 GRÜNBAUM. (*a*) Lancet, ii, pp. 806, 1559, 1745. 1896. (*b*) *Ibid.*, i, p. 451. 1897.

8 ISSAEFF. Ann. de l'Inst. Pasteur, p. 260. 1893.

9 LEVY u. BRUNS. Berl. Klin. Wochschr., p. 491. 1897.

10 METSCHNIKOFF. Ann. de l'Inst. Pasteur, p. 465. 1891.

11 PFEIFFER, R. Ztschr. f. Hygiene, vol. 18, p. 1.

12 SALIMBENI. Ann. de l'Inst. Pasteur, p. 277. 1897.

13 WASHBOURN. Journ. of Pathology, vol. 3, p. 214.

14 WIDAL. (*a*) Bull. de la Soc. Méd., p. 561. 1896. (*b*) Ann. de l'Inst. Pasteur, p. 353. 1897 (contains his collected papers and many references).

15 WRIGHT. (*a*) Lancet, ii, p. 809. 1896. (*b*) Brit. Med. Journ., i, p. 256. 1897.

NOTE ON THE SPECIFIC ACTION OF NORMAL HUMAN SERUM UPON THE BACILLUS COLI COMMUNIS*

By S. R. CHRISTOPHERS, M.B. Vict.

From the Pathological Laboratory, University College Liverpool

The application of the serum diagnosis to the differentiation of the typhoid bacillus from the various varieties of the bacillus coli communis has not hitherto been as satisfactory in its results as might have been anticipated.

Peckham† gives a table showing the action of typhoid human serum upon 70 cultures of typhoid and coli bacilli obtained from various sources, and lays stress upon the anomalous results obtained by her application of this test to the differentiation of the two forms. In her list the majority of cultures giving the biological characters of coli were undoubtedly agglutinated by human typhoid serum; one in particular she mentions as giving the reaction as fully, that is, clumping, and almost entire cessation of movement, as did any of the typhoid cultures.

Ziemke‡ states that whilst using cultures of coli as controls in the performance of the Widal test, he frequently found that they were agglutinated by typhoid human serum.

Bensaude§ also states that the agglutinins of typhoid serum act upon the B. coli, though in a less degree than upon the typhoid bacillus, and explains this action as being due to the close biological relationship of the two organisms.

Such marked action of typhoid serum upon the B. coli would naturally invalidate the strict specific nature of the reaction between typhoid serum and the typhoid bacilli. There is, however, a source of error, which I have not seen mentioned, which may be to a large extent responsible for these puzzling results. It is that many have used, as the differentiating test, the blood of human typhoids. Now, in the following table, it will be seen that normal human serum has, in the great majority of cases, as specific an action upon the bacillus coli communis as has the serum of a typhoid patient upon the bacillus typhosus; that, in fact, the serum of a typhoid patient probably has two distinct specific actions, one upon the typhoid bacillus due to infection by that organism, and one upon the B. coli, an action which is present alike in typhoid and non-typhoid sera.

Table I shows the action of a number or non-typhoid and typhoid sera upon the B. coli, with the amount of dilution with which the reaction could be obtained. The coli organism used

---

* Reprinted from the 'British Medical Journal.'

† The 'Journal of Experimental Medicine,' vol. ii, No. 5, 1897.

‡ Ziemke. 'Deutsche Med. Wochensch,' 8 April, 1897.

§ 'Le phénomène de l'agglutination des Microbes,' par Raoul Bensaude.

TABLE I

		Typhoid.	Cholera.	Coli.
Non-typhoid.	Normal	Not 1 to 5	Not 1 to 5	1 to 30
	Normal	„	„	1 to 120
	Normal	„	„	1 to 60
	Phthisis	„	„	1 to 30
	Diabetes	Signs 1 to 5	„	„
	Lead poisoning	Not 1 to 5	„	„
	Empyema	„	„	1 to 20
	Pleural effusion	„	„	1 to 60
	Atheroma	Not 1 to 5	Not 1 to 5	1 to 60
	Hemiplegia	„	„	1 to 30
	Normal	1 to 5	Signs 1 to 5	„
	Perityphlitis	Signs 1 to 5	Not 1 to 5	1 to 120
	Leucocythæmia	1 to 10	1 to 10	1 to 200
	Normal	Not 1 to 5	Not 1 to 5	Not 1 to 20
	Normal	1 to 10	„	1 to 60
Typhoid.	Fourth day	Not 1 to 5	Not 1 to 5	1 to 30
	Sixth day	1 to 60	„	„
	Sixth day	1 to 30	—	—
	Sixth day	„	„	1 to 20
	Seventh day	„	„	1 to 30
	Eighth day	1 to 120	„	1 to 60
	Tenth day	Not 1 to 5	„	1 to 30
	Thirteenth day	1 to 120	„	1 to 120

At the dilutions given the action was not pronounced negative until after two hours. At such small dilutions as 1 to 20 the coli organism was agglutinated within 20 minutes with precisely similar appearances to those seen in the agglutination of the typhoid bacillus.

was one which gave in a marked degree the characters of the B. coli, such as indol formation, coagulation of milk, gas formation, marked growth on potato, etc.

In order to see to what extent this action would hold good with different varieties of colon bacillus, I tried the action of normal and typhoid sera in dilution upon 16 cultures of supposed coli organisms taken from different sources. Three of these were acting pathogenically, 4 were from sewage, 4 from milk, 3 from mussels, and 1 from cheese.

It will be seen that the majority of these were agglutinated by human serum, independently of the fact whether it was from a typhoid patient or not. In fact, had any of these been suspected typhoid organisms, and the action of human typhoid serum been tried upon them, they might very easily have been returned as typhoid bacilli.

In face of these results, I think that in the use of human typhoid serum for diagnosing suspected typhoid bacilli found in water, milk, etc., the equally specific action of the serum upon B. coli must also be borne in mind.

In the examination of a number of rabbits' sera I found that, in the majority of cases, dilution to the extent of 1 in 20 did away with all agglutinating action upon both the typhoid and

TABLE II

	Pathogenic.					Sewage.				Milk.				Mussels.			Cheese.
	Typhoid from Spleen.	Lab. Coli.	Coli from Appendix.	Coli from Cystitis.	Coli from Diarrhœa.	C_1	C_2	C_3	C_4	C_1	C_2	C_3	C_4^5	C_1	C_2	C_3	C
Coagulation of milk		×	×	×	×	×	×	×	×		×	×	×				×
Gas formation		×	×	×	×	×	×	×	×	×	×	×	×	×	×		
Indol		×	×	×		×		×	×		×	×	×				
Marked growth on potato ...		×	×	×		×	×	×	×			×	×			×	
Human typhoid serum at dil. 1-20	×	×	×	×	×	×		×	×	×	×	×	×				
Human normal serum at dil. 1-20		×	×	×	×	×		×	×	×	×	×	×				
Normal rabbit serum at dil. of 1-40																	
Serum of typhoid injected rabbit at dil. of 1-40 ...	×																
Serum of coli injected rabbit at dil. of 1-40		×	×	×	×			×	×	×		×					

colon bacillus. Such animals injected with typhoid cultures ought (supposing a strict specific action) to give a serum agglutinating the typhoid bacillus and not the colon bacillus.

In Table II it will be seen that on none of the coli-like organisms had the serum of a typhoid rabbit (in dilution) any action, whilst it had a marked action on typhoid bacilli. So that, presumably, the results of those observers who have used the sera of injected animals as the differentiating agent have escaped the above-mentioned error.

However, even if injected animals be used, the serum ought to be proved to have no action upon the colon bacillus in the dilution at which it is proposed to use it as a differentiating test.

THE BACTERIOLOGICAL DIAGNOSIS OF PLAGUE*

By C. BALFOUR STEWART, M.A., M.B.Camb.

Thompson Yates Laboratories, University College Liverpool; late attached to Plague Research Laboratory, Bombay; Assistant Bacteriologist Liverpool Corporation; Research Assistant, Pathological School, Thompson Yates Laboratories

The following methods are those adopted at the Plague Research Laboratory for diagnosing plague in Man or animals, and for testing a plague culture. Naturally, in Bombay, we had to do with recent cultures, and the following remarks apply to such. A plague culture grown for a long time on artificial media does not seem to give the same results; at any rate, a culture of plague which had been kept for a long time in a laboratory, and which was given me to examine, did not grow typically in broth.

Media

Only two kinds of media are used—agar and broth; it is found impossible to work with gelatine on account of the high temperature. Ordinary agar slant tubes are allowed to dry. Should they be required for use soon after they are made it is best to place them for a few days in a drying bottle, with quicklime at the bottom, otherwise they may be dried by leaving them on their sides in a dark room for a few weeks. If plate cultures are made it is better to prepare the plate, and let it dry and make a smear culture.

Broth

A special peptone solution and infusion of meat made out of Goat's flesh is used on account of native religious prejudice against beef and commercial peptone, but ordinary broth made of beef infusion, *plus* 1 per cent. peptone *plus* 0.5 per cent. salt does just as well, or better.

The broth need not be neutralized unless very acid. I have often found the plague bacillus grow better in broth made without any addition of alkali, the reaction to blue litmus paper being a slight tinge of red. As such broth generally gives a precipitate after sterilization, and requires to be filtered and sterilized again, it is better for practical purposes to render the broth slightly alkaline, the less the better, and not more than is given by 0.2 gram per cent. of caustic soda added to the broth after being made neutral. About 100 c.cm. should be put into globular flasks of about 200 c.cm. capacity, and sterilized at a slightly lower temperature than that at which the broth was originally heated; if this is done, no further precipitate will come down.

Source of the Plague Microbe

The plague microbe is found in the blood shortly before a fatal termination of a case of plague, so the specimen should be taken shortly before or soon after death.

The sputum of a case of pulmonary plague contains the bacilli. If the bacillus is to be sought for in the bubo, it is better to make a small incision in the skin, and push a small sterilized glass pipette into the gland, and suck up a drop or two of the juice. This should be done before suppuration has commenced.

* Reprinted from the 'British Medical Journal.'

Temperature of Incubation

In Bombay it is found better not to use an incubator. The microbe grows best at the ordinary room temperature. By placing a large number of tubes to incubate, under varying conditions, I found the best growth on those incubated at 74° F. in a cupboard. In this country an incubator would be required.

Growth on Agar

The colonies appear in 24 to 48 hours. If numerous, they appear to the naked eye as small round points, colourless and translucent. Under Zeiss Obj., A they are translucent with slightly greenish tinge, granular, and have slightly ragged edges.

If the colonies are scanty the individual ones may grow larger. The growth takes place in successive concentric circles round the original colony, which appears heaped up in the centre and is more the colour of mother-of-pearl as it grows older.

A plague colony slips about on the surface of the agar when touched with a platinum wire.

If a dried agar slant tube is inseminated by introducing 0.1 to 0.2 c.cm. of a broth culture by means of a long thin glass pipette, and if the culture is smeared evenly over the surface with a thin glass rod or with the end of the pipette (care being taken not to dig into the agar), the so-called 'ground-glass' appearance is seen after one or two days' incubation. The layer of growth should be viewed from underneath through the substance of the agar with the light coming from opposite, the eye being almost level with the slanting surface of the agar tube held about a foot off. The layer of growth looks like bright ground-glass or like the back of a looking-glass. The agar must be dry so that it will absorb the broth leaving the bacilli on the surface. If there is any broth not absorbed, or any condensation water present, the growth in contact with the liquid is smeary and somewhat milky. After inseminating the tubes it is well to leave them on their sides for a few minutes and then incubate them in the upright position. In examining them care should be taken not to allow any liquid that may be at the bottom of the tubes to flow over the surface of the growth.

This 'ground-glass' appearance was first shown by Haffkine. It is a very useful method of testing the purity of a culture known to be plague, because a strange colony is generally detected at a glance. Some microbes, proteus for example, will grow in amongst the plague, when the film will no longer have a typical 'ground-glass' appearance, but will appear somewhat slimy.

Neither is this 'ground-glass' appearance peculiar to the plague microbe alone, for I succeeded in separating a spore-forming micro-organism from the air that showed the 'ground-glass' appearance after 24 hours' growth; but the growth became opaque after 48 hours' incubation, coincident with the formation of spores.

Broth

Haffkine considers the stalactitic growth of plague in broth to be characteristic of the microbe. He puts a drop or two of cocoa-nut oil or ghee (native clarified butter) in the flasks before they are sterilized; the oil globules form a thin broken film on the surface of the broth. After inoculation the flask must be incubated on a firm shelf or table. In three to six days numerous thin threads of growth are seen hanging down from the surface into the otherwise clear

broth. If the flask is gently shaken, the stalactites fall down to the bottom like a fall of snow; after a time the broth becomes clear again, and in a day or two another lot of stalactites will be found hanging from the surface.

It is most important to keep the flasks still, for the slightest movement or vibration is sufficient to cause the stalactites to drop. A shelf is most convenient to rest the flasks on, because the stalactites are best seen by placing a small candle-end lighted behind the flask and looking through it. If an incubator is used, the flask should be placed on the front of the shelf so as to allow room for the light behind, and the door should be opened very gently; an incubator would have to be kept specially for the purpose. The film of oil probably acts merely as a *point d'appui*; it is by no means necessary, but it hastens the formation of stalactites.

In making broth cultivations the writer always inseminated the flasks by means of a thin drawn-out glass rod long enough to project above the mouth of the flask; one end is held in the fingers, and the rod passed seven times slowly through the flame. The other sterilized end is drawn over the culture, the rod is then passed into the flask between the cotton wool plug and the glass till the end touches the bottom, the unsterilized part of the rod projecting above the plug. If the flask is observed day by day another characteristic phenomenon may be seen: the microbe growth creeps up the glass rod till it reaches the surface, and then gradually spreads out till the surface of the broth is covered with numbers of small round colonies, which grow down into the substance of the broth as 'stalactites.' A few whitish colonies may be seen growing on the bottom of the flask and up the sides; the broth itself remains perfectly clear.

This curious form of growth on the surface of the glass seems to be characteristic of the plague microbe; it proves a useful test, because some idea can be formed before the 'stalactites' have had time to grow. If the microbe is found to be growing in this way, and if the broth itself remains clear, so far as the writer's experience goes it has always turned out to be plague, and shows a 'stalactitic' growth in a few days more. No fat or oil was used in these cultivations.

Microscopic Examination

The plague microbe stains readily with aniline dyes, but not by Gram's method. The bipolar staining in stained specimens, when the ends are found to be deeper stained than the centre, is by no means a constant phenomenon and cannot be relied on for diagnosis. Mr. Watkins-Pitchford considers it due to a greater collection or condensation of the protoplasm in these regions; he notes that the spots are readily visible in the living and unstained specimens, so they cannot be due to staining. The detection of the spots he considers to be a most valuable aid to the rapid identification of the germ. Dr. E. L. Marsh drew my attention to the fact that plague microbes suspended in water dry as a bluish film on a microscopic slide; if he does not get this he considers it unnecessary to go on with the staining.

The bacillus is polymorphic; it is sometimes seen as a very short bacillus, almost like a coccus or diplococcus; at other times as a short stumpy bacillus with rounded ends. Very often it is found in pairs as a diplobacillus, and sometimes in distinct short chains. I have found this latter form chiefly in broth, especially in the unneutralized broth.

PRELIMINARY NOTE

ON SOME EXPERIMENTS TO DETERMINE THE COMPARATIVE EFFICACY OF THE DIFFERENT CONSTITUENTS OF HAFFKINE'S PLAGUE PROPHYLACTIC *

By C. BALFOUR STEWART, M.A., M.B. Camb.

The following short note gives the results of experiments done at the Plague Research Laboratory, Bombay, between February and May, 1899. The full account has been sent in to Government, and will appear in due course.

A number of Rabbits were inoculated with increasing doses of a young culture of living plague in broth, and the somewhat unexpected result was found that the Rabbits which received the larger doses showed the smaller mortality. I thought it possible that during the growth in broth the latter had acquired sufficient prophylactic properties to render the animal immune to the microbes contained in the broth culture injected.

To see whether the prophylactic property lay in the broth altered during the growth of the microbe or in the sediment, several sets of experiments were done in which the broth was filtered through a porcelain filter. The sediment of a certain quantity of living plague culture was found to be more virulent than the same quantity of broth culture unfiltered when injected into Rabbits.

Some Rabbits were inoculated with the filtrate alone, others with the sterilized sediment alone, and immediately afterwards injected with a suspension of living plague from an agar tube. The result seemed to show that both the filtrate and the sterilized sediment possessed prophylactic properties.

Immunity seems to be conferred even when the animal is inoculated with the prophylactic immediately before the injection of plague.

In testing the virulence of a plague culture, it is evidently important to use only a very young broth culture or an agar culture.

---

* From the Plague Research Laboratory, Bombay.

EXPERIMENTS TO DETERMINE THE EFFICACY OF THE DIFFERENT CONSTITUENTS OF HAFFKINE'S PLAGUE PROPHYLACTIC*

By C. BALFOUR STEWART, M.A., M.B. Camb., Thompson Yates Laboratory, University College Liverpool

In Haffkine's anti-cholera inoculations in India it was noticed that, whereas the case incidence among the inoculated was much reduced, the case mortality was not influenced; the inoculations produced a resistance against the invasion of the body by the cholera microbe, but no resistance against the toxins formed if they once caused infection.

Gamaleia showed that it was possible to immunize an animal against virulent microbes without it being immune to the toxins of the same microbe produced artificially in the laboratory. Behring and Kitasato showed that by inoculating an animal with increasing doses of toxin it could be rendered resistant to a lethal dose.

Haffkine acted on these observations in devising the plan of anti-plague inoculations. He says: 'When, in 1896, I was confronted with the problem of working out a prophylactic treatment against the plague, I determined to put to the test the ideas originated by the observation on our cholera patients, and to attempt, in the new preventive inoculation, to obtain at once a lowering of the susceptibility to the disease and a reduction of the case mortality. This I resolved to obtain by treating the system with a combination of bodies of microbes and of concentrated products of them. In giving the above considerations I beg that they should be considered as temporary, subject to modifications or to complete refutation.'†

The following experiments, of which a preliminary note appeared in the 'British Medical Journal' for September 2nd, 1899, were done at the Plague Research Laboratory, Bombay, to try and supply experimental proof to this theory.

I had previously remarked that when several rabbits were inoculated with varying doses of a ten-day-old living broth culture of plague those that received the larger dose showed the lesser mortality. Arguing that when the larger dose was given, a quantity of certain substances capable of conferring immunity or reducing the severity of the case were injected along with the living microbes, and that since a young culture was used and the microbes presumably all living, this substance was probably contained in the broth acted upon by the growth of the microbes, I proceeded to inoculate a series of rabbits with increasing doses of living plague culture in broth.

In some previous experiments I had found that about 2 c.cm. of Haffkine's prophylactic per 1,400 grams of Rabbit were sufficient to give immunity. In these experiments a smaller dose was taken because immunity was not wanted; what I wanted to find was whether there was any

* 'British Medical Journal,' March 3rd, 1900.

† A Discourse on Preventive Inoculation. Delivered at the Royal Society, Sweden, on June 8th, 1899, by W. M. Haffkine, C.I.E.

benefit in the larger dose of metabolised broth. Moreover, if the Rabbits became ill it would be from plague, since the culture was not sterilized; and if more recovered after receiving the larger dose than was the case with those receiving the lesser it would show that the larger dose of metabolised broth tended to lower the case mortality.

Seventeen Rabbits were inoculated with a broth culture of plague from the laboratory stock. Letter *P* denotes that a post-mortem examination was made and the plague bacillus recovered from the heart blood.

EXPERIMENT I

No.	Weight.									Result.
1	2,020	grams	inoculated	with	0.175	c.cm.	8	days old	living culture	Survived.
2	1,480	„	„	„	0.25	„	„	„	„	Died in 64 hours. *P.*
3	1,910	„	„	„	0.5	„	„	„	„	Survived.
4	1,470	„	„	„	0.75	„	„	„	„	Died in 20 hours. *P.*
5	1,540	„	„	„	1.0	„	„	„	„	Died in 64 hours. *P.*
6	1,810	„	„	„	1.25	„	„	„	„	Survived.
7	1,750	„	„	„	1.5	„	„	„	„	Survived.

EXPERIMENT II

No.	Weight.									Result.
8	1,000	grams	inoculated	with	0.5	c.cm.	8	days old	living culture	Died in 75 hours. *P.*
9	1,050	„	„	„	0.75	„	„	„	„	Died 6th day. No *P.*-*M.*
10	1,300	„	„	„	1.0	„	„	„	„	Died in 60 hours. *P.*
11	1,450	„	„	„	1.25	„	„	„	„	Died in 60 hours. *P.*
12	1,280	„	„	„	1.5	„	„	„	„	Survived.
13	1,480	„	„	„	1.5	„	„	„	„	Survived.

EXPERIMENT III

No.	Weight.									Result.
14	1,750	grams	inoculated	with	0.5	c.cm.	11	days old	living culture	Died 6th day. No *P.*-*M.*
15	990	„	„	„	0.5	„	„	„	„	Died 6th day. No *P.*-*M.*
16	1,880	„	„	„	1.5	„	„	„	„	Survived.
17	940	„	„	„	1.5	„	„	„	„	Died 3rd day, 10 hours. *P.*

As a control to these, some of the culture that was used for the last four Rabbits was filtered through a porcelain filter, and the microbes were washed off the candle into a quantity of sterilized water equal in amount to the broth culture taken. Four Rabbits were inoculated.

No.	Weight.						Result.
18	1,420	grams	inoculated	with	0.5	suspension	Died 4 days 20 hours. *P.*
19	900	„	„	„	0.5	„	Died 5 days 20 hours. *P.*
20	1,290	„	„	„	1·5	„	Died 3 days 23 hours. *P.*
21	840	„	„	„	1.5	„	Died 3 days 22 hours. *P.*

Thus, out of 10 Rabbits inoculated with doses of 1 c.cm. and under of living plague broth culture, 8 died; and of 7 Rabbits inoculated with doses over 1 c.cm. and up to 1.5 c.cm., only 2 died. It is evident that the larger dose contained sufficient active substance to reduce the mortality, and as the cultures were young it is more likely the substance was contained in the broth than in the deposit, which would consist almost entirely of living microbes.

Another experiment was made in which the supernatant fluid in the broth acted upon by the microbe was separated from the sediment of microbes, and the two constituents tested separately and together with respect to their prophylactic properties.

Experiment IV

A 21-day-old broth culture, obtained recently from a patient, was filtered through a porcelain filter, and the microbes remaining on the candle of the filter were washed off into a quantity of sterilized water, equal in amount to the broth of the culture taken. The suspension was heated at 60° C. for thirty minutes. Rabbits were inoculated as follows :

No.	Weight.					
1	...	1,750	grams	inoculated with	3	c.cm. filtrate.
2	...	1,150	,,	,,	3	,, ,,
3	...	1,150	,,	,,	3	,, suspension.
4	...	1,610	,,	,,	3	,, ,,
5	...	1,150	,,	,,	0.75	,, filtrate, 0.75 c.cm. suspension.
6	...	1,150	,,	,,	0.75	,, ,, 0.75 ,, ,,

Immediately after they were inoculated in the other flank with $\frac{1}{8}$ of a suspension of a 3-days-old agar culture of plague obtained from the same broth culture. Two controls were also inoculated with the same quantity of the living culture alone. The controls both died, one in forty-eight hours, the other in three to four days ; a large number of bacilli were found in the heart blood.

Nos. 1 and 2 both survived, but No. 1 was ill for 6 days.

No. 3 died in 3 days, but very few bacilli were found in the heart blood, either by the microscope or by cultivation.

No. 4 survived.

No. 5 died in 49 hours, but no plague microbes were found in the heart blood, so it cannot be said to have died of plague.

No. 6 survived.

It appears from this experiment that the filtrate alone conferred power to prevent or to modify an attack of plague ; Rabbit No. 1 probably had plague, but survived.

The suspension of sterilized microbes seems to have conferred bactericidal properties, for the blood of the Rabbit No. 3 contained few bacilli compared to that of the controls.

The mixture of filtrate and suspension of sterilized microbes protected Rabbits Nos. 5 and 6 from plague infection, for no plague bacilli were found in the blood of Rabbit No. 5. The dose of the mixture was equivalent to 0.75 c.cm. of culture ; this dose was taken and not 1.5 c.cm., as appeared from the first experiments to be required, because in this last experiment older, and therefore stronger, culture was used.

Lustig and Galeotti* state that the immunizing property is a nucleo-proteid contained in the bodies of the microbes, and that if Haffkine's prophylactic has any immunizing property it is due to the nucleo-proteid dissolved or still contained in the bodies of the microbe. But this would not obtain in the above experiments, for the cultures were made in slightly acid broth—that is, ordinary beef broth without the addition of an alkali, so the nucleo-proteid would not be dissolved. Moreover, the cultures used were young and the microbes probably all living and not in a state to give up their nucleo-proteid.

---

* 'British Medical Journal,' February 10th, 1900.

These observers advocate a prophylactic made of a solution of the nucleo-proteid obtained from microbes grown on solid media, and they say that HAFFKINE concurred with their view when he tried to test the strength of his liquid by the quantity of deposit produced in the cultures. This no doubt is so, for inasmuch as the bactericidal property is conferred by substances contained in the dead bodies of the microbes, he naturally tests the strength of the prophylactic by the amount of deposit. The primary object is to produce a prophylactic substance; but HAFFKINE, on theoretical grounds, thought the supernatant fluid, by virtue of the toxins, conferred power to withstand the toxins formed if the persons did contract plague after being inoculated—that is to say, it reduced the case mortality; for these reasons he preferred to keep the supernatant fluid in his prophylactic.

The foregoing results show experimental confirmation of the theory. I recognise that many more observations should be made. I used up all the Rabbits procurable in Bombay at the time, and was unable to obtain any more, but I hear that these experiments have subsequently been repeated and confirmed by Captain MILNE, I.M.S., at the same laboratory.

A NEW MICROCOCCUS

WITH A NOTE ON THE BACTERIOLOGY OF LYMPHADENOMA*

From the Pathological Laboratory, University College Liverpool.

By JOHN HILL ABRAM, M.D., M.R.C.P., Assistant Physician, Royal Infirmary; Senior Demonstrator of Pathology, University College Liverpool

Plate XII

I have lately had a case of lymphadenoma under my care, in which I have made a bacteriological examination of the blood during life. I have long felt that the ordinary method of obtaining blood for this purpose is unsatisfactory, and I adopted the following method, which was, I believe, first recommended by Sittmann. The skin of the forearm was sterilized by packing with a towel soaked in 1 to 1000 perchloride of mercury for two hours, and then the needle of a hypodermic syringe (sterilized by boiling for five minutes) was thrust into one of the superficial veins. In this way a fair quantity of blood can be obtained without causing more than momentary pain.

Plates were made in the usual manner. Fourteen colonies developed in a single plate, the majority being pure growths of *Staphylococcus albus*. In addition to these there were three colonies of an organism having the following characteristics :—

Medium.	Incubat. Temp.	
Gelatine plate - -	21° C.	Slow growth. Colony circular, surface finely granular, margin regular. Slow liquefaction.
„ stab - -	...	Growth faster at surface than in depth. Slow liquefaction reaching wall of tube in 6 days.
Agar plate - -	37° C.	Fairly rapid growth on surface, colony round, regular edges.
„ stroke - -	...	Growth fairly rapid, is slimy and resembles a streak of white paint.
Potato - - -	...	Good growth. Culture raised, dark red, no staining of potato substance.
Milk - - -	...	No coagulation.
Broth - - -	...	Abundant growth ; powdery sediment.
Litmus agar - -	...	Medium turns red.
Glucose gelatine -	21° C.	No gas formation.

Microscopically the organism is a small micrococcus, aggregated in clumps. It stains well by the ordinary methods ; it is not stained by Gram's method ; it is non-motile. Inoculation

* From the 'Journal of Pathology and Bacteriology.'

experiments have given negative results. So far as I can ascertain the organism isolated has not been described before.

Further examinations of the blood have failed to detect any organisms, and, taking into account the failure of the inoculations, it is evident that this organism has nothing to do with lymphadenoma. At the time it was present the patient was undergoing a febrile attack, no doubt due to the *Staphylococcus albus* infection.

Before considering the question of the bacteriology of HODGKIN's disease, it is necessary to point out the confusion which exists with regard to the exact application of the term lyphadenoma. This is well shown in the list of names given by VON LIMBECK* under the generic term—Pseudo-leukæmia: (HODGKIN's disease), anæmia lymphatica (WILKS), cachexie sans leucémie (BONFILS), adenie (TROUSSEAU), anæmia splenica (GRIESINGER), malignes lympho-sarcom (LANGHANS), malignes lymphom (BILLROTH), desmoid carcinom (R. SCHULZ).

It is, I believe, not yet possible to draw sharp lines between the conditions which are mustered under the title of pseudo-leukæmia, but it seems probable that it includes more than one disease.

Taking the condition from a general point of view, it has of late years been more and more frequently urged that it is of an infective nature, on the score of (1) the anatomical structure of the enlarged glands, and the deposits in the various organs; (2) the symptomatic features, anæmia, hæmorrhages, and so forth.

On looking up the literature, the investigations of the disease from the infective point of view have not been numerous. KLEBS† states that WEIGERT found bacilli having the staining characteristics of tubercle bacilli in the enlarged mesenteric glands, and that he himself in one case found a short, freely motile bacillus in the blood, both during life and post-mortem. KELSCH and VAILLARD‡ in a case of 'Tumeurs lymphadeniques multiples avec Leucémie,' found once during life and in the organs after death a short non-motile bacillus, hardly longer than broad, staining readily with methylene-blue, but not by GRAM's method. It did not liquefy gelatine, and on potato at 35° C. gave a moist shining yellowish-brown growth. Inoculations into Guinea-pigs gave no results, but Rabbits and white Mice were killed in 36 hours. They do not claim specific properties for this organism.

DRESCHFELD§ has put on record a case of acute HODGKIN's disease, in which small bacilli were found in the kidney post-mortem. Cultivations were not obtained. TRAVERSA‖ has recorded a case of acute pseudo-leukæmia, in which streptococci were found in the blood during life, and GROSSI¶ a similar case, in which the examination had a negative bacteriological result. LANNOIS and GROUX** have recorded a case of acute pseudo-leukæmia in which *Staphylococcus pyogenes aureus* was found.

It may fairly be assumed that, in my case and in those of TRAVERSA, LANNOIS, and GROUX,

* Von Limbeck. 'Klin. Path. des Blutes,' 2 te Aufl. S. 326.
† Klebs. 'Allgemeine Pathologie.'
‡ Kelsch and Vaillard. 'Ann. de l'Inst. Pasteur,' Paris, 1890, tome iv, p. 276.
§ Dreschfeld. 'Brit. Med. Journ.,' London, 1892, vol. i, p. 893.
‖ Traversa. 'Riforma med.,' Napoli, 1893, t. ix, p. 26.
¶ Grossi. *Ibid.*, 1893, t. ix, p. 62.
** Lannois and Groux. 'Lyon méd.,' 1890, No. 34.

PLATE XII.

Fig 1

Fig 2

Fig 3

Fig 4

the organisms found were the result of a secondary infection ; and in my own case it is interesting to note that, during the period of secondary infection, the glandular swellings diminished greatly in size, to again enlarge when the organisms disappeared from the blood. In DRESCHFELD's case the organism was found only in the kidney, and moreover, only after death, so that but little value can be attached to its presence. KELSCH and VAILLARD claim no specific property for their organism.

In conclusion, the organism of lymphadenoma, if organism there be, has yet to be isolated, and the method of SITTMANN would appear to afford the best means for its detection.

DESCRIPTION OF PLATE XII

FIG. 1.—Agar growth.
FIG. 2.—Potato growth.

MOUSE FAVUS*

By JOHN HILL ABRAM, M.D., M.R.C.P., Assistant Physician, Royal Infirmary;
Senior Demonstrator of Pathology, University College Liverpool
From the Pathological Laboratory, University College Liverpool.

Plate XII

In the spring of 1897 a Mouse was sent to the laboratory for examination. It was stated that all the Mice caught in a local sugar refinery were in a similar condition to the one we received. On examination a thick greyish crust was obvious, extending from the mouth backwards over the cheek on to the trunk.

I had considerable difficulty in obtaining pure cultivations, but eventually succeeded by adopting Kral's method. A portion of the crust was rubbed down with sterilized sand in a sterilized mortar, the resulting mixture distributed in melted agar, and plates made in the usual manner.

In this way pyogenic cocci were isolated, and an organism which had the following cultural characteristics :—

Gelatine at 21° C. - -	Growth slow, with gradual liquefaction.
Agar at 37° C. - -	Growth fairly rapid. The centre is occupied by a dense mass, which does not penetrate deeply into medium; the margin shows an abundant feathery outgrowth. After some days the centre of the growth becomes pink and eventually deep red.
Tartaric acid agar - -	Growth more rapid than on agar. Less marginal growth, but marked superficial growth of low, thick, white aërial hyphæ. Red colouration also more marked.
Tartaric acid potato - -	Fair growth. Surface covered with dense growth, pure white in colour. The growth has an appearance exactly resembling the icing on a cake.

Examination of the feathery marginal growth shows a thickly growing branching mycelium, with rounded swellings in the course and at the ends of the hyphæ. No special fructification organs can be made out.

Inoculations upon Mice and Rats produced typical favus growths. Inoculation on a Hen failed.

Nicolaier, in Flügge's laboratory, has investigated a similar condition, and isolated a fungus which gave cultures in all respects similar to mine. Inoculations also gave identical results.

I have made cultivations on similar media of a stock human favus organism, obtained from the favus of Man, with the result that after repeated inoculations upon the tartaric acid media, the growth became almost identical with that of the mouse favus organism.

* From the 'Journal of Pathology and Bacteriology.'

The absence of any definite fructification organ makes the differentiation of the two organisms almost impossible, but for my own part I believe them to be identical. This opinion is also held by BOER.*

DESCRIPTION OF PLATE XII

FIG. 3.—Agar growth.
FIG. 4.—Tartaric acid potato.

* 'Vrtljschr. f. Dermat.,' Wien., Bd. lxxxvii, S. 429.

TUBERCLE BACILLI IN MILK, BUTTER, AND MARGARINE*

By H. E. ANNETT, M.D. Vict., D.P.H., Professor Koch's Institute, Berlin, and the Thompson Yates Laboratories, Liverpool

In the midst of the great movement at present occupying the attention and interest both of the profession and the public, namely, the provision of sanatoria and homes for the phthisical, there is some danger of the fact that tuberculosis is a preventable disease being considerably neglected. The human digestive tract plays an important, but probably the most easily preventable, rôle in infection by tubercle bacilli; a rôle, however, insignificant in comparison to that of the respiratory tract in tuberculosis of adults, but very important and considerable in the case of young children and infants. In view of the most recent experimental work on the question of the presence of tubercle bacilli in milk, butter, and margarine, an apology for again introducing this subject, on which much has already been written, is deemed unnecessary.

Tubercle Bacilli in Milk

Numerous bacteriological examinations of milk during the last ten years have proved the presence of tubercle bacilli in a greater or less percentage; for example, in Berlin milk, by Obermüller,† to the extent 61 per cent., and by Petri,‡ also in Berlin milk, only of 14 per cent.—using the method of inoculation of animals as the test. Also Rabinowitsch and Kempner§ found that 28 per cent. of 25 samples of Berlin milk contained tubercle bacilli.

In the medical officer of health's report‖ to the Liverpool Health Committee on the question of tubercle bacilli in the milk-supply of Liverpool, 2.8 per cent. of the 144 samples collected from sources within the city were proved to be infective by experiments of Professor Sims Woodhead, Professor Boyce, and Professor Delepine; while 29.1 per cent. of 24 samples taken on arrival from parts of Cheshire, Shropshire, &c., at the railway stations were found to be tuberculous. Similarly Professor Delepine¶ reports of the milk-supplies of Liverpool, Manchester, and other parts, 5.55 per cent. of 54 samples collected from town dairies; and 17.6 per cent. of 125 samples of milk from country farms collected at the railway stations proved to be tuberculous. Again, milk collected from 16 Cambridgeshire dairies, a sample from each, was reported by Kanthack and Sladen** to have produced tuberculosis in Guinea-pigs, on inoculation, to the extent of 56.3 per cent. of the milk (9 samples).

---

* Reprinted from the 'Lancet,' Jan. 20th, 1900.
† 'Hygienische Rundschau,' 1895, No. 19.
‡ 'Arbeiten aus dem Kaiserlichen Gesundheitsamte,' 1898, vol. xiv.
§ Rabinowitsch and Kempner: 'Zeitschrift für Hygiene,' 1899, p. 137.
‖ 'Report and Brit. Med. Jour.,' 1897, vol. ii, p. 162.
¶ 'Brit. Med. Jour.,' 1898, vol. ii, p. 917.
** The 'Lancet,' Jan. 14th, 1899, p. 74.

Professor BOYCE, to whom I am indebted for permission to use his results, has carried on numerous experiments during the last two years for the Health Committee of the Corporation of Liverpool. During the year 1898, 84 samples of 'town' milk, of which 7 (= 8.3 per cent.) proved to be tuberculous on Guinea-pig inoculation; and 28 samples of 'railway' or 'country' milk, of which 5 (= 17.8 per cent.) were infective, were examined; while during 1899, to the end of June, of 75 samples of 'town' and 63 samples of 'railway' milk, 6.6 per cent. of the former and 17.4 per cent. of the latter proved to be tuberculous. These results indicate the effects of better sanitation in the cowsheds, shippons, and dairy premises in large towns, as compared with country places, enforced by local by-laws, ensuring better ventilation, spacing, cleanliness, and regular inspection.

The consideration of the relation between the presence of tubercle bacilli in milk, and the extent of the disease in the animal yielding that milk, adds greater and more important interest to the question. The use of tuberculin for the detection of tuberculosis in cattle seems to be an almost infallible test. The presence of a small tuberculous lymphatic gland has often proved, post-mortem, to be the only lesion occasioning the reaction to tuberculin; indeed, some investigators are so sure of its infallibility that they assert that, although no naked-eye lesions may be detected at the usual examination, a careful search will discover deposits in parts generally never looked at—spinal column, joints, and interior of bones, &c.

In 1880 BOLLINGER,* by inoculation experiments, found the milk of a Cow with tuberculosis of the udder to be infective, as also that of another case of tuberculosis without lesion of the udder. MAY† obtained only one positive result with the milk of six 'graped' Cows, and STEIN‡ four positive results from 14 'graped' Cows. BANG,§ of Copenhagen, has made numerous experiments. The milks from 63 Cows with advanced tuberculosis were proved to be tuberculous only in 14 per cent. HIRSCHBERGER‖ asserts from his experiments (20 cases with 11 positive results) that milk may be infective when only a small lesion occurs in the lung. ERNST¶ examined 114 samples of milk from 36 tuberculous Cows showing no udder leison and 28.57 per cent. proved to be infective. SMITH and SCHRÖDER** found the milk infective in 2 cases out of 6 tuberculous animals with no udder lesion; and SCHRÖDER, later, found the same in two samples of milk from 31 tuberculous cases. DELEPINE†† proved the presence of tuberculosis in the milk of 2 cases out of 6 which had reacted to tuberculin and showed, clinically, more or less evidence of the disease. In these two cases the udders showed lesions microscopically. Later he examined the milk of 24 suspicious udders with results shown in his table reproduced :—

---

* 'Aerztliches Intelligenzblatt,' 1880, p. 409.
† 'Archiv für Hygiene,' 1883, Band i, p. 121.
‡ Inaugural Dissertation, Berlin, 1884.
§ 'Deutsche Zeitschrift für Thiermedicin und Vergleichende Pathologie,' 1884 and 1891, p. 1.
‖ 'Archiv für Klinische Medicin,' 1889.
¶ 'American Journal of the Medical Sciences,' November, 1889.
** 'United States Department of Agriculture, Bureau of Animal Industry :' Bulletin 3, 1893, p. 60; also Bulletin 7, 1894, p. 75.
†† 'Journal of Comparative Pathology,' 1897, p. 192.

TABLE SHOWING RESULTS OF MILK FROM 24 COWS WHOSE UDDERS WERE SUSPICIOUS

Milk from udders.	No. of samples.	Producing tuberculosis.		
		Actual number.	Percentages.	
A. Certainly diseased ...	10	5 } 6	50.0	} 31.5
B. Probably diseased ...	9	1 }	11.1	
C. Healthy	5	—	—	—
Total	24	6	—	—

RABINOWITSCH and KEMPNER'S* researches were most complete. Fifteen Cows were tested with tuberculin giving a distinct reaction. Three months later samples of their milk were tested, and that of 10 of them (66.6 per cent.) proved to be tuberculous. At the time the milk was taken, and previously, clinical examinations of the animals were made, and, in cases of death, post-mortem examinations. The results were as follows:—(*a*) Only one Cow showed distinct tuberculous udder lesions clinically (besides lung symptoms); her milk (two samples) proved to be infective. (*b*) One Cow showed, microscopically, only tuberculosis of the udder (clinically and post-mortem advanced disease of organs); the milk was found to be tuberculous. (*c*) Three Cows showed advanced general tuberculosis, the udder histologically exhibiting chronic interstitial inflammation without tuberculous deposits; the milk of all three was found to be tuberculous. (*d*) One Cow with lung lesions and some emaciation; the milk was found to be tuberculous. (*e*) One Cow showed symptoms of commencing lung lesions; the milk proved to be tuberculous. (*f*) One Cow on first examination was suspected; on two subsequent examinations the animal seemed normal; the milk was found to be tuberculous. (*g*) Two animals showed no symptoms on three examinations extending over a period of five months, but their milk proved to be tuberculous. (*h*) Four others, three showing clinical signs and one none, gave non-tuberculous milk (four, two, two, and two samples respectively). (*i*) In one other case, exhibiting post-mortem acute and chronic lesions, the inoculation experiments on Guinea-pigs failed from the presence of a pathogenic coccus. Therefore, omitting the last case, 10 out of 14 animals (71.4 per cent.), all of which had reacted to tuberculin, yielded tuberculous milk. More experimental work is evidently desirable; but one must clearly infer from these experiments that any animal which reacts to tuberculin indicating lesions which, may be, cannot, even by repeated clinical examination, be detected, is capable of giving milk, which will produce tuberculosis in the Guinea-pig on inoculation.

In contrast to RABINOWITSCH'S results OSTERTAG† concludes, from experiments with the milk of 50 animals which reacted to tuberculin but showed clinically no sign of disease, that the milk of such animals is absolutely non-infectious for Guinea-pigs.

---

* 'Zeitschrift für Hygiene,' 1889, Band i, p. 145.

† 'Zeitschrift für Fleisch- und Milchhygiene,' 1899, p. 221.

Tubercle Bacilli in Butter

It has been proved by many authors that butter made from tuberculous milk was also infective; and by Heim* and Gasperini† that tubercle bacilli live and keep their virulence in butter for a period of even 12 days.

Brusaferro‡ found virulent tubercle bacilli in 1 sample out of 9; Roth§ in 2 samples out of 20; Schuchardt‖ only in 1 out of 42 samples; and Gröning¶ in 8 (47 per cent.) out of 17 samples.

From the results of experiments by Rabinowitsch** it is now doubtful whether the animals in the above-mentioned experiments (references 19 to 22) were infected with Koch's bacillus tuberculosis. She examined 30 samples of butter from Berlin and 50 from Philadelphia, and *in no single case* found bacilli 'which, by means of culture and the pathological results of Guinea-pig inoculations, could be pronounced as identical with Koch's bacillus tuberculosis;' but '23 samples (28.7 per cent.) produced changes which, both macro- and microscopically, resembled those of tuberculosis, but which by more extensive and exact examination could be distinguished with ease.' Other authors have described similar pseudo-tuberculosis organisms; Petri†† in milk, Moeller‡‡ in milk, in 'Timotheus' grass, and in fæces of cattle. The characteristics of Rabinowitsch's micro-organism are as follows:—It is immotile, and in form closely resembles the bacillus tuberculosis. The bacilli generally occur singly and are often slightly curved; but when growing rapidly in tissue bacilli are often found lying parallel. Sometimes they form long unbranched threads, and sometimes are divided into short pieces. The bacilli are somewhat thicker than bacillus tuberculosis and often show a club-shaped swelling on one side. Spores are not formed, but one portion of the bacillus stains often more intensely than the rest. The bacilli stained by many methods of staining tubercle bacilli cannot be distinguished from the latter; only by the employment of very dilute watery solutions of methylene blue could any distinguishing feature be observed, viz., that bacilli from a culture of bacillus tuberculosis stain only at one spot, the rest of the bacillus remaining unstained; while in the case of bacillus pseudo-tuberculosis the whole bacillus stains faintly and generally uniformly, seldom showing a more deeply-stained part.

Cultural differences, however, occur. On agar, the bacilli taken direct from an infected animal produce visible colonies on the second or third day. At first the agar surface is covered with a thick, moist, creamy layer: in old cultures by a folded membrane often orange- or copper-coloured. After repeated passages through animals cultures on agar or glycerine-agar show a dry, brittle, crumpled membrane resembling that of bacillus tuberculosis. In plate cultures the deep colonies are grey in colour, round or oval, and uniformly granular. On the surface colonies are better developed, have a uniformly granular grey centre, and a clear wavy outer zone; the surface of the colony is often dry and conical. On butter-agar in fresh cultures the colonies are small,

---

* 'Arbeiten aus dem Kaiserlichen Gesundheitsamte,' 1889, Band v, p. 294.
† 'Giornale della Reale Società d'Igiene,' 1890.
‡ 'Giornale di Med. Veter. Prat.,' 1890, p. 201.
§ 'Correspondenzblatt für Schweizer Aerzte,' 1894.
‖ 'Inaugural Dissertation,' Marburg, 1896.
¶ 'Centralzeitung für Veterinär-, Viehmarkts-, und Schlachthof-Angelegenheiten,' 1897, Nos. 14 and 15.
** 'Zeitschrift für Hygiene,' 1897, p. 90.
†† 'Arbeiten aus dem Kaiserlichen Gesundheitsamte,' 1898, Band xiv.
‡‡ 'Therapeutische Monatshefte,' November, 1898.

white, and dry, later spreading over the whole surface and becoming yellow or copper-coloured. On potato a luxuriantly-growing, moist, grey layer is formed. In gelatine growth proceeds very slowly at ordinary room temperatures, colonies becoming visible on the 3rd day. In broth, and especially in glycerine broth, growth is rapid, forming in 2 or 3 days a folded membrane on the surface, the broth remaining clear, the culture closely resembling that of bacillus tuberculosis. Broth cultures are distinguishable from those of bacillus tuberculosis by their characteristic odour, being unpleasant and ammoniacal; that of bacillus tuberculosis being agreeable and resembling the odour of flowers. A small quantity of indol is formed in broth cultures, which is not so in bacillus tuberculosis cultures. Milk is not coagulated, and on the surface is an abundant yellowish-red layer which clings firmly to the glass. On albumin-free colourless media a growth appears in 2 or 3 days, becoming in 10 days a thick yellow folded membrane; bacillus tuberculosis in the same time on such media forming a thin layer just covering the surface and just beginning to fold. The presence of fat in these bacilli can easily be demonstrated as in the case of bacillus tuberculosis.

Pathogenic Properties of Bacillus Pseudo-tuberculosis

The following are the post-mortem appearances observed in a Guinea-pig killed three or four weeks after intraperitoneal injection of butter containing the bacillus pseudo-tuberculosis. There is a slightly distended abdomen; also peritonitis, with adhesions varying in nature from delicate fibrinous bands to firm connective tissue. The peritoneum and mesentery are studded with nodules. The mesenteric glands are swollen and may contain purulent or caseous matter. The liver is covered with nodules and patches which may be raised above the liver substance or may penetrate into the liver parenchyma. The spleen is sometimes only enlarged, at other times thickly studded with nodules. The kidneys show yellowish patches. The lungs are covered with small transparent nodules which do not penetrate into the lung tissue. The sternal lymphatic glands are swollen, but show no caseation. Numerous bacilli can be demonstrated in these lesions. Many animals after injection show considerable signs of illness during the first 14 days with diminution in weight and then recover. Pure cultures of this bacillus are only pathogenic for Guinea-pigs (but not always); Rabbits and white Mice are immune. After intraperitoneal inoculation of Guinea-pigs some die in from 4 to 8 weeks with considerable emaciation and show the following post-mortem appearances; others survive. At the seat of inoculation there is a purulent infiltration containing the characteristic bacilli; also peritonitis, varying in intensity from a flocculent fibrinous exudation to strong connective tissue adhesions, The mesentery is studded with small nodules; the glands are enlarged but not caseating; there are patches on the liver, and miliary nodules throughout an enlarged spleen. The thoracic cavity and organs are often almost exempt from lesions. Infected animals do not react to tuberculin. Histologically the nodules in the liver and spleen consist of a collection of lymphoid elements with but very few epithelioid and multinuclear cells. The bacilli are found in the middle of a young nodule, towards the periphery if caseation has commenced. The typical giant cells of tuberculosis do not occur. More often—especially after inoculation with butter containing the

pseudo-tuberculosis bacilli—the nodules appeared to consist of a central necrosed portion surrounded by leucocytic infiltrated area.

Tubercle Bacilli in Margarine

Margarine was first prepared by M. Mege Mouries. In his process 1,000 kilogrammes of ground fat from freshly slaughtered cattle is mixed with 300 kilogrammes of water, one kilogramme of potassium carbonate, and two sheep's or pig's stomachs cut into pieces, and is heated at 45° C. by steam for 2 hours. The fat rises and, after being skimmed, is run off and heated at from 30° to 40° C. with 2 per cent. of salt, the clear fat being then poured off from the sediment and cooled at from 20° to 25° C. The granular solid product is cut into pieces, packed into linen, and exposed to hydraulic pressure at about 25° C., with the production of a solid cake of stearin and a liquid oleomagarine, which is passed through cylinders and washed by a shower of water. Of the melted oleomargarine 50 kilogrammes are mixed with 25 litres of cow's milk and 25 kilogrammes of water in which has been macerated 100 grains of finely ground mammary gland of the cow. Annatto is added and the whole churned for about 2 hours, and the product kneaded and washed.

In the method employed in the United States, Austria, and some parts of Germany, pepsin is not added, the orginal finely-ground fat being subjected to a temperature below 50° C. for some time. The oleomargarine is mixed with sour milk and a small quantity of bicarbonate of soda and annatto and is churned. After about 15 minutes it is cooled in ice and then kneaded to eliminate the water, then returned with more sour milk, and is ready for sale. In Holland the oleomargarine is melted at about 50° C., and is mixed with the requisite amount of milk and of the best Kampen butter and earthnut (arachis), and other oils (cotton, palm, cocoanut, &c.), to lower the melting-point to that of butter. The Kampen butter is prepared specially for this manufacture from an unskimmed mixture of milk and cream, and is preferred on account of its powerful flavour which, though unpleasant alone, imparts a pleasant flavour to the margarine.

Hence margarine may be infected in three ways—(1) from the original cattle (tubercle bacilli survive even a temperature of 55° C. for almost 4 hours); (2) from the butter and milk added; and (3) from contamination during manipulation (phthisical workmen, &c.). Few researches as to the presence of tubercle bacilli in margarine had been done before the year 1899. Morgennoth,* of the Hygienic Institute, Berlin, early last year examined 20 samples of margarine and in 9 cases proved the presence of virulent tubercle bacilli of Koch—a very large percentage (45 per cent.).

In October, 1898, in Berlin, I commenced researches on this question. Thirty-six samples of margarine were collected from shops in different parts of Berlin. Two Guinea-pigs received 5 cubic centimetres each from each sample intraperitoneally, the margarine having been just melted at from 45° to 50° C. and injected semi-fluid. Of the 36 samples, 21 caused death of both Guinea-pigs inoculated within 10 days; 9 others caused the death of 1 only of the pair—from peritonitis or septicæmia So that 15 samples only could be considered, 9 with 1 animal each,

* 'Hygienische Rundschau,' November, 1899.

6 with 2. These were all killed some 8 or 10 weeks after inoculation; but with 1 sample only, and that in both the Guinea-pigs injected, could there be found any lesion indicating the presence of tubercle bacilli. In these were nodules on the mesenery containing caseous material; enlarged purulent mesenteric glands; the intestines and organs were adherent to each other and to the abdominal wall; the liver was enlarged and studded with several dirty yellow patches penetrating into the liver tissue; the spleen was adherent but apparently normal (it was enlarged in one case); and the lungs were dotted on their surfaces with small transparent nodules. The lymphatic glands and nodules on the liver contained bacilli in large number, staining by ZIEHL-NIELSEN's method. Cultures failed because of rapid overgrowth of accompanying micro-organisms. Subcutaneous inoculation into Guinea-pigs from the lymphatic glands and liver nodules gave rise to abscesses which evacuated and healed, bacilli staining by ZIEHL-NIELSEN being demonstrated in the pus. These animals, killed some weeks after, showed no further lesion. In sections of the nodules in the organs of the original Guinea-pigs the *typical* tuberculous characteristics (giant cells and epithelioid cells) failed. There lesions were therefore probably occasioned, not by KOCH's bacillus tuberculosis, but by an organism identical or similar to that described by RABINOWITSCH and detailed above.

In Liverpool, early in 1899, the experiments were continued with 13 samples collected from various shops in the city. Subcutaneous inoculation was practised, 2 Guinea-pigs for each sample. In this case only 2 Guinea-pigs died within 10 days; so that 2 samples had only 1 animal each. Out of these 13 samples only 1 proved to be tuberculous, the Guinea-pig exhibiting, post-mortem, the typical characteristic, both macro- and microscopically, of real tuberculosis. Inoculations from the lesions subcutaneously were followed by typical tuberculosis in Guinea-pigs. Thus, out of a total of 28 samples, 15 of Berlin and 13 of Liverpool margarine, only one proved to contain virulent bacillus tuberculosis of KOCH, and that in a sample from the latter city.

I wish to take this opportunity of thanking my late colleagues at Professor KOCH's Institute for their great courtesy and kindness towards me during the 18 months when I worked among them, and particularly Professor KOCH, Professor PFEIFFER, Professor BRIEGER, and Professor FROSCH for their help and advice during my researches.

A CLASSIFICATION OF THE MICRO-ORGANISMS FOUND IN WATER*

By RUBERT W. BOYCE, M.B., Professor of Pathology, University College Liverpool

and

CHARLES A. HILL, M.B., B.C., B.A. Cantab., Assistant Bacteriologist, University College Liverpool

From the Pathological Laboratory, University College Liverpool.

The following are briefly the results we have obtained from a year's constant examination of the Liverpool waters. The conclusions we have arrived at tend largely to support those arrived at by Professor Marshall Ward in his fifth report to the Water Research Committee of the Royal Society. Our observations are based upon 400 analyses.

The sources of the waters examined are the following:—(1) The Streams and Lakes of the Watershed; (2) the water of the Reservoirs before filtration; (3) the water of the Reservoirs after filtration; (4) the water in the Mains and Service Reservoirs; (5) the water in the Dead Ends; (6) the water in the Wells; (7) the bacteria present in the Sand of the filter beds.

The water has always been collected in small sterilized bottles, or in vacuum bulbs, which have been broken under the surface of the water. Plate cultures on gelatine and agar-agar were made as soon after collection as possible, and in most cases on the spot.

Photographs were prepared of all the characteristic plate cultures, and it is hoped these may serve to aid in the more rapid identification of colonies. The photographs showed clearly that different bacterial floras exist for different localities.

Results

1. *Water in Rivulets of Watershed.*—There is a marked preponderance of the *Bacillus fluorescens liquefaciens.*

2. *Water of Reservoirs before filtration.*—There are considerable numbers of liquefying bacilli, namely, *B. fluorescens liquefaciens*, *B. luteus liquefaciens*, and the *Proteus* group.

3. *Water of Reservoirs immediately after filtration.*—There is a marked elimination of the liquefying forms; the chief forms present being exceedingly slow-growing forms, and consisting of non-liquefying cocci and bacilli, chromogenic and non-chromogenic. It is probable that many of these forms are derived from the sand in the process of filtration, and are more especially water bacteria.

---

* Reprinted from the 'Journal of Pathology and Bacteriology,' May, 1899.

4. *Water in the Mains and Service Reservoirs after filtration.*—The three principal liquefactive groups mentioned above again make their appearance; the *B. fluorescens* being in excess over the other two.

5. *Water in the Dead Ends.*—There are almost pure cultures of various groups of bacteria; thus, in one case, the *B. violaceus* largely predominated; in another case, a very beautiful iridescent bacillus; in a third, a white liquefying organism giving rise to a pearl-like appearance in the gelatine.

6. *Water in the Wells.*—Our investigations so far show chiefly slow-growing, non-liquefactive types.

7. *The bacteria present in the Sand of the filter beds.*—In addition to the slow-growing bacteria previously mentioned, the *B. ramosus* and *B. violaceus* were constantly found.

In addition to the bacteria proper we have isolated a very characteristic group of higher forms, allied to the streptothrix and saccharomyces, and other forms of fungi, which produce various pigments, such as dark brown, pink, and white, and which emit a very characteristic and pungent odour, recalling the smell of damp cellars. In addition to photographing the plates, all the new colonies were isolated and inoculated on the various media, and their action studied on glucose gelatine, in broth, and in milk.

We have thus formed a living herbarium, which at present numbers 115 specimens, although some of these may be found to be duplicates showing slight variations. We consider that the grouping adopted by Professor MARSHALL WARD to be far more satisfactory than trying to identify the bacteria with the innumerable forms described in various text-books.

In the following classification we have adopted the group numbers of Professor MARSHALL WARD, having been readily able to identify these, and we have added some extra groups to include types not described in his list.

GROUP 2.—*Bacilli producing a violet pigment.*—Seven members of this group were studied in detail. They all produce a blue pigment, varying in intensity from a light blue amethyst to a deep bluish-black, which is developed only after several days' incubation. After prolonged subculture in the laboratory they tend to lose their power of pigment production, and to become white. The optimum temperature is about 20° C. No growth takes place at 37° C.

On gelatine their liquefactive action varies, and is, as a rule, limited and slow. In milk they grow readily, and produce the characteristic pigment; the medium becomes slightly acid, and in some instances a pellicle forms on the surface. The casein is not precipitated.

In glucose gelatine no gas is produced. In peptone salt solution the pigment is produced, and often a pellicle on the surface. No formation of indol was noted.

GROUP 3.—*Fluorescent liquefying bacilli.*—Six members of this group were studied in detail. They are distinguished by their smell and rapid liquefactive action on gelatine, and the formation of a greenish fluorescent pigment on agar and gelatine. They grow readily at all temperatures between 20° and 38° C. The casein in milk is rapidly precipitated, and subsequently completely peptonized with a slightly acid reaction. In glucose gelatine no gas is produced. In peptone salt solution they grow readily; no indol is produced.

GROUP 4.—*Fluorescent non-liquefying bacilli.*—Six members of this group were studied in detail. They show a marked resemblance in many details to the previous group, and may be merely an enfeebled form of that species, more especially as the power of liquefying gelatine is often regained on subculture, though entirely absent on isolation.

GROUP 5.—*Colourless non-liquefying bacilli.*—Twenty organisms were isolated and studied in detail which could be referred to this heading. With one exception all showed variations from the typical cultural characteristics and reactions of the colon bacillus. For instance, many of them were incapable of precipitating casein in milk, though acidifying the medium; only about half the number were able to produce gas in glucose gelatine, but one formed indol in peptone salt solution. They all grew readily on gelatine, without liquefying it, and on potato formed a well-marked yellowish growth.

GROUP 6.—*Common Proteus type.*—Six forms were isolated and studied. One of the most marked features of this group, besides the 'wandering islets,' is the putrid smell produced by growth in milk and broth. Gelatine when liquefied by their action also acquires a sour odour. We have observed no variability in the liquefying power. Milk, as a rule, is acidified, and the casein precipitated and peptonized. The formation of gas in glucose gelatine is not constant.

GROUP 7.—*The yellow Proteus type.*—Six forms were isolated and observed. They were closely allied to the last group, and only differ in that they form a well-marked yellow pigment.

GROUP 8.—*A group which may be identical with Ward's Group* 8.—Bacilli, forming irregular colonies of a beautiful iridescent appearance. Two members of this group were isolated for observation. They slowly liquefy gelatine. On agar and potato they form a yellow pigment. The casein in milk is precipitated and peptonized with a faintly acid reaction. No gas is formed in shake cultures. No action on broth. They resemble in many particulars the preceding and the following group.

GROUP 9.—*Golden-yellow liquefying bacilli.*—Seven organisms were isolated and studied in detail; they differ in rapidity of liquefaction, but all form a golden-yellow pigment, and closely resemble Group 7 —the yellow *Proteus* type—in their action on milk and glucose gelatine.

GROUP 10.—*Golden-yellow non-liquefying bacilli.*—Five members of this group were isolated for observation. They are slow-growing forms, and are probably, as MARSHALL WARD suggests, weakened varieties of the previous group, as their liquefactive action is frequently regained on subculture, while their behaviour in milk is not constant.

From our observations on Groups 6 to 10 inclusive, we are in agreement with Professor MARSHALL WARD's conclusions, namely, that these are merely varieties of one or two species, and that it is impossible to draw any hard-and-fast distinctions between the several groups.

GROUP 13.—*Bacilli producing a crimson pigment.*—Two forms of this type were isolated and studied in detail. Cultures have a crimson-red metallic sheen with a marked trimethylamine smell. Gelatine is rapidly liquefied. No loss of liquefying power was observed. The casein in milk is precipitated and peptonized with an acid reaction, whilst later the tube contents acquire a crimson hue. In glucose gelatine gas is produced in forty-eight hours, whilst broth acquires a marked crimson tint.

GROUP 15.—*Bacilli conforming to the type of B. subtilis.*—Thirteen organisms were isolated and kept under observation. From their study we have come to the conclusion that it is impossible to distinguish between the 'hay bacillus' and the 'potato bacillus'; they are probably varieties of the same species. Under this heading we have included also well-known types, such as *B. ramosus* and *B. megaterium*, which from their behaviour on subculture conform closely to this type.

The liquefactive action on gelatine is fairly constant, though some members tend to lose it on prolonged cultivation. The formation of a pellicle on the liquefied gelatine and in peptone salt solution is a distinguishing feature.

The wrinkled growth on potato is common to all the members of this group, and cannot be relied upon to differentiate them.

In milk the casein is rapidly precipitated and peptonized with the formation of varying degrees of acidity. The formation of gas in glucose gelatine is rarely observed.

GROUP 16.—*Sarcinæ forming a yellow pigment.*—Two varieties were isolated and kept under observation. Members of this group produce a yellow pigment of varying intensity, which tends to decrease on prolonged subculture. The liquefactive action on gelatine varies considerably, as does their peptonizing action on milk. The true sarcina form of this group is not always maintained.

GROUP 19.—*Non-chromogenic micrococci.*—Eleven organisms were isolated and studied in detail. Both liquefying and non-liquefying forms were met with, but this property is not constant, and in fact was found to be interchangeable. The microscopical appearances show wide differences, some members

closely simulating a yeast, others streptococci ; but these forms are not constant, and tend to disappear on prolonged subculture. Some forms have a peptonizing action on milk, but others are of so feeble a growth that no change takes place.

In addition to the above we have isolated organisms which we have been unable to classify under any of Professor MARSHALL WARD's groups. We have found it necessary, therefore, to include them under a fresh grouping. These are the following :—

GROUP A.—*Chromogenic micrococci.*—These differ from his Group 17 in that they form a marked yellow pigment of varying degrees of intensity. They are very common. We have studied 16 forms in all. Their liquefactive action on gelatine, like that of Group 19, is not constant, some losing it on prolonged subculture, others regaining it. Many of them are feebly-growing forms, especially on potato, and have a tendency to become non-chromogenic. A few are capable of peptonizing milk. None of them were found to be capable of producing gas in glucose gelatine.

GROUP B.—In addition there is the very characteristic group mentioned above, allied to the cladothrix, streptothrix, saccharomyces, and other fungi, of which we have isolated 5 examples. These produce varying coloured pigments—such as dark brown, pink, and white—and a most pungent odour, recalling the smell of a damp cellar. They all produce liquefaction of gelatine, and an acid reaction in milk, but no gas in glucose gelatine. On potato white chalky growth forms, having the characteristic smell, and an appearance like bird excrement.

GROUP C.—Lastly, we have isolated a pure yeast form, which produces a pink colour and liquefies gelatine, whilst precipitating the casein in milk with an acid reaction. It does not produce gas in glucose gelatine.

PARLIAMENTARY POWERS

FOR THE SANITARY SUPERVISION AND CONTROL OF ICE-CREAM MANUFACTURE*

By E. PETRONELL MANBY, M.D., D.P.H.

Assistant Medical Officer of Health, Liverpool, Lecturer in Public Health, University College Liverpool

That the manufacture and storage of ice-cream is, as a rule, carried out under very insanitary conditions is no new fact. The question of interest to Medical Officers of Health is how these conditions, so prejudicial to health, can be improved. The subject has been discussed on more than one occasion at one society or another, and resolutions condemning the present state of affairs and urging the granting of controlling powers to Health Authorities have been passed. As far as I am aware, however, Liverpool is at present the only Local Authority in England which has obtained such Parliamentary powers. Perhaps, therefore, it may be of some interest to you to hear what those powers are, and our experience of the first year's working under them.

Several outbreaks of typhoid fever, apparently due to contaminated ice-cream, have been reported of late years.

A full account of such an outbreak appeared in Dr. Hope's Annual Report on the Health of Liverpool for 1897, and I will only very briefly mention the points here.

All the patients were children of school age, and all attended the same school.

Possible sources of infection at the homes of the patients were excluded, and attention was of course at once directed to conditions of the school, but everything was found satisfactory there.

The period of illness of the various patients indicated the end of the first week in September as the probable date of infection.

Inquiry elicited the fact that a village fair had been held in Knotty Ash, a suburb of Liverpool, on September 6th and 7th, and that every one of the children suffering from typhoid had visited the fair. An ice-cream and chip potato vendor was present, and all the children but three admitted that they had partaken of his ice-cream. Of these three two had eaten only chip potatoes and no ice-cream, and the other was too young and too ill to give any account of its proceedings.

It was then discovered that two children living at New Brighton (*i.e.*, some miles from Liverpool on the other side of the Mersey), but who had been at the Knotty Ash fair and eaten the ice-cream there, were lying ill at home with typhoid fever, and a few days later identical information was received with reference to a school boy at Denbigh.

---

* Reprinted from the 'Journal of the Sanitary Institute,' vol. xx, part iv.

These children had not attended, nor had they even entered, the Knotty Ash school.

Instructions were issued to the Sanitary Inspectors to visit every ice-cream establishment within the municipality of Liverpool, to ascertain whether or no any person in connection with them had been to the Knotty Ash fair, and if any sickness existed at their houses about that time.

The result of this investigation brought to light the fact that a certain Italian vendor of ice-cream and chip potatoes had been at the fair, and had his wife sick in the house at the time: which sickness proved to be typhoid fever. It was the second case in the house, the first having been notified and removed to hospital on August 11th.

That, therefore, was as far as we could get: no bacteriological examination of the ice-cream supposed to be the cause of the outbreak being possible at this distance of time. I think, however, you will agree with me that the chain of evidence was as complete as it was possible to be. The premises of the Italian were kept in a dirty state, and knowing the habits of these people, we can easily imagine how the ice-cream became infected.

Professor BOYCE, the Corporation Bacteriologist, examined a sample of ice-cream from the shop of this Italian, and his report on it, as on many other specimens afterwards taken in various parts of the town was, 'teeming with bacteria. Infinitely worse than ordinary milk which has stood for some time.' Professor BOYCE adds: 'Some of the colonies grown on agar closely resemble forms which I have obtained from samples of sewage.'

In due course Dr. HOPE reported to the Health Committee that 'the conditions of this Italian's premises may be taken as typical of the conditions existing in many places where ice-cream is made for hawking in the streets. Powers for the registration and supervision of such places are urgently needed. In the meantime, inasmuch as milk is an ingredient in the manufacture, the places where ice-cream is made will be dealt with in exactly the same manner as places where milk is sold.'

An omnibus bill for Liverpool was about to be laid before Parliament, and in it powers for *licensing the persons* selling, and *registering the premises* used for the manufacture, storage, and sale of ice-cream and similar commodities were asked for.

For some reason the House of Commons Committee did not seem to view these proposals with favour. Many objections were raised, but in the end the clause approved of by the Committee and passed by Parliament was as follows:—

'32.—(1) Any person being a manufacturer of or merchant or dealer in ice-creams or other similar commodity who within the City—

(*a*) Causes or permits ice-creams or any similar commodity to be manufactured sold or stored in any cellar or room in which there is an inlet or opening to the drain; or

(*b*) In the manufacture sale or storage of any such commodity does any act or thing likely to expose such commodity to infection or contamination or omits to take any proper precaution for the due protection of such commodity from infection or contamination; or

(*c*) Omits on the outbreak of any infectious or contagious disease amongst the persons employed in his business to give notice thereof to the Medical Officer of Health for the City :

shall be liable for every such offence on summary conviction to a penalty not exceeding forty shillings.'

The clause, though good as far as it goes, does not, it will be seen, give the Local Authority power to license the vendor as in the case of the Dairies and Cowsheds Order, nor to register the premises as in the case of the Liverpool Improvement Act, 1867, nor does it provide powers of entry for the purposes of inspection, &c.

At the same time, although there is no compulsion to be registered, the power possessed by the Health Authorities is really very little affected by this omission.

If the premises had to be registered, the Health Department would not do this unless the arrangements were satisfactory. At present, if the people apply, and are told their premises are unsuitable, or even if they make no application, if the Health Authorities consider that they are unsuitable, without further trouble an information can be laid, and proceedings taken under Section 32 of the Act. We usually warn the people once or twice to desist from carrying on the manufacture, and then, if they persist, we lay an information in the ordinary course. Soon, however, when the provisions of the Act become generally known, this 'warning' will not be necessarily a preliminary to legal action.

The Liverpool Corporation Act, 1898, of which we are speaking, received the Royal assent on August 12th, 1898.

Since that date to August 12th, 1899, 319 persons have applied directly or indirectly to the Health Department for permission to sell ice-cream ; 56 persons have been told that, owing to unsuitability of premises, &c., they should make other arrangements. 1,600 visits have been paid by the two Inspectors who look after this work. Three informations have been laid, two cases heard before the stipendiary magistrate, and two convictions obtained with fines of 20s. and 40s. respectively, and costs in each case. One case was withdrawn.

The stipendiary has in each case pointed out the serious character of the offences, remarking once that the best punishment for the defendant would be to make him live on his own ice-cream for a time.

We have not thought it requisite to make any special regulations for the ice-cream trade. We regard it as a form of milk-selling, and carry out the new regulations applicable to that business made by the Corporation of Liverpool in 1897.

The procedure of the Health Department is briefly this :—The address of the premises being secured—whether by the occupant applying to the Health Office, or from information obtained in other ways—a visit is paid by one of the Inspectors, and a report is made of present conditions, and the possibility or otherwise of adapting the premises for the purpose intended, also of the repairs, cleaning, &c., required.

If necessary Dr. Hope or I then visit the premises, and the occupier is afterwards formally told whether they are or are not suitable.

A large amount of ice-cream is, of course, sold in shops supplying sweets, mineral waters, and other refreshments throughout the City. Most of these are already registered as milkshops, are under constant supervision, and, as a rule, require very little doing to them before commencing or continuing the sale of ice-cream.

Besides these, however, there are two other classes of manufacturers and vendors :—

(1) Large businesses for the wholesale manufacture of the material for supplying, for example, passenger steamers, hotels, and the like.

(2) Small traders, mostly, though by no means only, Italians, who as a rule manufacture and store the ice-cream and the ingredients of which it is composed under grossly insanitary conditions. Some of these traders have only a barrow trade ; others do that and a retail shop business too—often combining the sale of fried fish and chip potatoes with that of ice-cream and other 'delicacies.'

With regard to these two classes, our aim has been to get the large manufacturer either to make his present premises thoroughly sanitary, or, failing that, to take new ones. In the case of the small trader, to get him to combine with others in the same business and to take some suitable building where they can all manufacture and store their ice-cream, instead of using their own houses.

In a very large number of cases this has taken place, but where for personal or other reasons a trader prefers to keep his business entirely to himself, he must then strictly conform to the regulations.

In conclusion, I would venture to suggest that pending the passing of a general Act preventing the manufacture and storage of ice-cream under insanitary conditions, for which Act I fear we shall have to wait a long time, each Local Authority in whose district any considerable trade of this kind exists should apply for Parliamentary powers on its own account on the lines of, and, if possible, better than those granted by the Liverpool Act of 1898.

The reader of the paper concluded by describing and exhibiting plans of two typical premises where ice-cream was being manufactured under insanitary conditions, and of the buildings to which the offender in each case moved, as a result of the representation of the Health Department.

MEAT INSPECTION*

AND THE ABOLITION OF PRIVATE SLAUGHTERHOUSES

Being a Paper read in the Section of State Medicine at the Sixty-seventh Annual Meeting of the British Medical Association held in Portsmouth, August, 1899.

By E. PETRONELL MANBY, M.D., D.P.H.

Assistant Medical Officer of Health, Liverpool, Lecturer on Public Health, University College Liverpool

Introduction

I am well aware that little that is new can be said on this subject. It has been discussed on many occasions and in many places. Why, therefore, should one tell again a twice-told tale? I will give you at once the reason I have in mind in bringing the subject before the State Medicine Section of the British Medical Association.

It is because at a great gathering like the annual meeting very many medical men are present whose direct connection with public health work is slight. That is to say, the majority of them are not medical officers of health—a condition the reverse of what obtains at sanitary congresses. So that, either at the meeting itself, or through the medium of our valuable 'Journal,' one is to some extent addressing an audience many of whom, perhaps, have not fully considered the subject of meat inspection, nor the need in England for improvement in such inspection. Now these medical practitioners are in touch with thousands of thinking men and women throughout the country, and have infinite powers of forming and influencing public opinion on health questions, if they will only use them—powers far greater, perhaps, than they recognize.

The medical officer of health is primarily concerned with his health committee, and cannot, except secondarily, influence private individuals. The health committee may approve the reports of their medical officer, and the town council may confirm the views of the health committee, but behind and far above both is the central governing body of the country—Parliament—which is not always influenced—I say it in all humbleness—by the opinion of experts, but by its master, the 'free and independent elector.'

No great reforms, sanitary or otherwise, are brought about in this country by a stroke of the pen of a high official. They come by the educating of public opinion. Let us, then, lose no time in educating public opinion aright on this meat question; for as an evidence of educating power misdirected—that is perverted education—the recent action of Parliament with regard to vaccination will occur to every one of us.

---

* Reprinted from the 'British Medical Journal,' Sept. 2nd, 1899.

All this, no doubt, is simply a statement of truisms; but what is it that the medical practitioners of the country can do in the way of bringing about an improvement in our system of meat inspection, and in the conditions under which animals intended for human food are slaughtered? It is by continually impressing on their friends and patients, influential and otherwise, for we all have votes nowadays, the need—the pressing need—in this country for improvement in the system. The abolition or private slaughterhouses, where only too often meat is prepared and kept under grossly insanitary conditions, and the substituting of public slaughterhouses under municipal control, being the only satisfactory means of attaining the desired end.

(*a*) The Commission reported on this question as follows:—

In favour of this view I may here quote from (*a*) the Report of the Royal Commission on Tuberculosis; (*b*) the Report of the Public Health Committee of the London County Council, July, 1898; (*c*) the recently published Harben Lectures of the Medical Officer of the Local Government Board, Sir Richard Thorne.

1. We recommend that in all towns and municipal boroughs in England and Wales, and in Ireland, powers be conferred on the authorities similar to those conferred on Scottish corporations and municipalities by the Burgh Police (Scotland) Act, 1892—namely:

(*a*) When the local authority in any town or urban district in England and Wales and Ireland have provided a public slaughterhouse, power be conferred on them to declare that no other place within the town or borough shall be used for slaughtering, except that a period of three years be allowed to the owners of existing registered private slaughterhouses to apply their premises to other purposes.

(*b*) That local authorities be empowered to require all meat slaughtered elsewhere than in a public slaughterhouse, and brought into the district for sale, to be taken to a place or places where such meat may be inspected; and that local authorities be empowered to make a charge to cover the reasonable expenses attendant on such inspection.

(*c*) That when a public slaughterhouse has been established, inspectors shall be engaged to inspect all animals immediately after slaughter, and stamp the joints of all carcasses passed as sound.

2. It appears desirable that in London the provision of public in substitution for private slaughterhouses should be considered in respect to the needs of London as a whole, and in determining their positions regard must be had for the convenient conveyance of animals by railway from markets beyond the limits of London, as well as from the Islington market, to the public slaughterhouses which should be provided. At the present time no administrative authority has statutory powers authorizing it to provide public slaughterhouses other than for the slaughter of foreign cattle at the port of debarkation.

3. With regard to slaughterhouses in rural districts the case is not so easy. But the difficulty is one that must be faced, otherwise there will be a dangerous tendency to send unwholesome animals to be slaughtered and sold in small villages where they will escape inspection. We recommend, therefore, that in Great Britain the inspection of meat in rural districts be administered by the county councils.

4. We recommend that it shall not be lawful to offer for sale the meat of any animal not killed in a duly licensed slaughterhouse.

(*b*) The Public Health Committee of the London County Council in July, 1898, submitted a report to the Council in the following terms:—

(*a*) That in the opinion of the Council it is desirable that, as a first step towards ensuring the proper inspection of meat, private slaughterhouses should cease to exist in London, and that butchers should in substitution be afforded such facilities as are necessary for the killing of animals in public slaughterhouses to be erected by the Council.

(*b*) That a copy of this report and of the Council's resolution thereon be sent to the Local Government Board, with an intimation that the Council is prepared to accept such responsibilities as may be necessary to give effect, in London, to the recommendations of the Royal Commission on Tuberculosis;

and that the Board be asked whether they will include in any legislation introduced by them in connection with the Royal Commission's Report the provisions which would be necessary for this purpose.

The Council, however, owing no doubt to the powerful opposition of the Meat and Cattle Trade Associations, has shelved the matter for the present.

(*c*) Sir Richard Thorne says :—

How is the very proper demand of the butchers for uniformity in the conditions regulating the seizure of carcasses on account of tuberculosis to be met? How is such skilful handling of slightly tuberculous carcasses to be attained as will secure the removal of the diseased portions in such a way that no risk shall attach to the remainder? I only know one answer, namely, by the abolition, as far as practicable, of private slaughterhouses, by the provision in all large centres of population, whether technically styled urban or rural, of public slaughterhouses under the direct control of the sanitary authorities and their officers, and by the adoption of measures which will, as soon as practicable, provide a class of skilled meat inspectors.

The properly administered public slaughterhouse is demanded as an act of justice to those trading in meat; it is demanded in the interests of public health and decency; it is demanded for the prevention of cruelty to the lower animals, and it is demanded in order to bring England, if not the United Kingdom, somewhat nearer to the level of other civilized nations in this matter.....

Public slaughterhouses, officered by skilled inspectors, and supervized by medical officers of health, are urgently required, amongst other reasons, for the prevention of tuberculosis in man.

Private Slaughterhouses

It is obvious to anyone how inefficient meat inspection must be where there are a large number of private slaughterhouses in a town. The occupiers naturally slaughter at times to suit their own convenience, and not that of the inspector. In Liverpool, with a population of 668,000, we have, besides an abattoir owned by a private company, only 30 private slaughterhouses, and we have five meat inspectors who devote their whole time to the work. But in smaller places where there are perhaps many more slaughterhouses and no special inspectors, meat inspection becomes very often practically a dead letter. One remedy suggested for this state of things is to increase the number of meat inspectors, as being cheaper than closing all private slaughterhouses and building municipal ones. Cheaper it would be, of course, and better than the present conditions, but how far behind the methods adopted, for example, in Germany.

And even if, as has been suggested, one inspector to every ten slaughterhouses be appointed, the question of insanitary conditions under which slaughtering takes place in many private slaughterhouses would not be remedied, though it might be improved.

Regulations in Foreign Countries

Both in arrangements for slaughtering and in meat inspection, England is very far behind when compared with continental countries, as those of us who have studied the subject in Berlin, Leipzig, and other places know well. In these towns well-managed municipal slaughterhouses exist—all private ones have been abolished; and all meat, whether killed at the abattoirs or imported, must be inspected and stamped by competent inspectors before being sold. In Germany laws were passed in 1868, 1876, 1879, and 1881 dealing directly with the question of the provision of public slaughterhouses, the prohibition of the erection of private slaughterhouses, and other matters connected therewith. These are the general slaughtering acts, and when the town

council of a place desire to apply them to their own town they obtain a local act giving them the necessary powers to build public slaughterhouses, close private ones, make regulations for the examination and stamping of meat, etc.

In Leipzig, for example, which possesses one of the most modern abattoirs in Germany :

1. No private slaughterhouses exists.

2. All slaughtering is carried on in the public slaughterhalls under sanitary surroundings, and the work of meat inspection is enormously facilitated thereby.

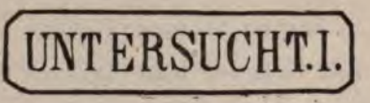
3. All meat brought into the town has to be taken to the central office for inspection before being sold.

4. Microscopical and other examinations of meat are carried out.

5. All meat passed as sound is stamped in several places with the official stamp showing that it is fit for human food.

6. Bad meat is taken away and destroyed by the municipality.

[I pass round a card showing the stamps used at Leipzig, and the arrangements in other towns are practically identical.]



The stamp of the inspector who passes or returns the meat.

Stamp showing that the meat is from the municipal abattior and not imported.

Stamp placed on imported meat which is passed.

The question of the Freibank should be mentioned, and though it has many points in its favour I do not think that English people at present would take to the idea. I have, however, seen much very fair meat sold at the Freibank at 4½d. per 0.5 kilo. (= 1.1 lb.). Practically no frozen New Zealand or River Plate meat is sold in Germany, and Freibank meat seems somewhat to replace it.

In Berlin, and in slaughterhouses of old construction, the system of separate small slaughterhouses, each let to one butcher, obtains. In Leipzig, Hanover, Cologne, Potsdam, and all modern buildings of the kind in Germany, the system of large slaughtering halls exists. In these no special place is assigned to any butcher, he just uses the space vacant at the moment.

[I also pass round a plan of the slaughterhouses at Leipzig, and have marked with a red ring the three slaughtering halls used respectively for cattle, swine, and sheep.]

The advantages of the large halls are that the systematic inspection of all carcasses and offal can be much more readily carried out, and that free ventilation and plenty of light are more readily obtained. Every director I questioned was in favour of the large halls, which, indeed, as I say, are universal in all new abattoirs in Germany now.

Stamp placed on imported meat which is sent back as not of good enough quality to pass though not bad enough to destroy.

Stamp placed on meat sent to the Freibank from the slaughterhouse.

It may be interesting to quote some of the regulations in force at Leipzig with regard to inspection, etc., of cattle slaughtered there, and of meat brought in from outside. This will indicate in the fewest words how complete the system is.

REGULATIONS MADE BY THE LEIPZIG COUNCIL AS TO THE EXAMINATION OF CATTLE AND MEAT

The examination of cattle, etc., brought into the market and slaughterhouses shall be made by experts appointed by the Council—namely, the director, two veterinary surgeons, six assistant veterinary surgeons, the chief of the microscopical department, the microscopical assistants, and the officers engaged in taking the samples of meat.

The director is the supreme officer of the establishment.

To him are delegated all powers for the examination of meat. All other employés are under his control.

He also exercises final judgment in all cases of dispute.

One of the two veterinary surgeons supervises the cattle markets, and also takes charge of the examination of all imported fresh meat. He is the director's deputy, and has the title of Official Cattle Market Veterinary Surgeon.

The other veterinary surgeon has the supervision of the slaughterhouses and the sanitary department, and is known as the Sanitary Veterinary Surgeon.

A veterinary surgeon is appointed to supervise each of the three slaughterhalls.

A veterinary or assistant veterinary surgeon shall inspect all animals entering the cattle markets and lairages.

Animals found to be suffering from disease shall be taken to the sanitary department for observation or slaughter.

All animals shall be inspected immediately after slaughter by the veterinary inspector who is present in the slaughterhall.

An additional microscopical examination of the carcasses of pigs, with a view to the discovery of trichinosis, shall be made by the examiners.

If, upon examination, the veterinary inspector finds the meat to be good and the intestines healthy, he shall mark each carcass with the veterinary stamp in a noticeable and conspicuous position, and shall order further marks to be placed on the meat by the superintendent of the slaughterhall.

As soon as a carcass has been marked the owner can dispose of it as he thinks fit.

Meat which is healthy but of poor quality, calves which are immature, also meat found unhealthy but which may still be eaten—all of which meat is wanting in nourishment—shall be marked as being of less value and of inferior quality and shall be transferred to the Freibank.

Where animals are found on slaughtering to be affected in a small degree, such as with abscesses, deformities, etc., having no influence on the general quality of the meat, the affected parts shall be taken away and the remainder of the carcass declared by the veterinary surgeon as fit for food.

Meat which, upon examination, is found to be unfit for human food shall be taken to the sanitary slaughterhouse, there to be destroyed in the usual way.

Examination of Imported Meat

(Meat which is not slaughtered in the city).

All meat which is brought into the city shall be taken, before being sold or eaten, to the inspection office at the public slaughterhouses, to be examined.

The person bringing meat for inspection shall give at the inspection office the name of the importer and butcher, and a full description of the meat.

If the meat is emaciated, or immature, or if there are any symptoms of disease, it shall be condemned and destroyed.

The meat shall be examined microscopically if necessary.

A register shall be kept of all meat which has been imported.

Meat which is submitted for examination shall not be removed from the inspection office until it has been stamped.

The imported meat, if good, shall be stamped as such, but with a different stamp to that which is placed on good meat slaughtered in the city, in order that all concerned may see that such meat is imported.

If the imported meat is offered for sale raw the purchaser must be informed in some way that it is imported meat.

Where the two kinds of meat are sold in one shop they shall be kept separate and sold for what they are.

Imported meat shall not be transferred to the Freibank.

Regulations as to Stamping of Meat

The meat which has been passed as fit for human food shall be stamped as follows :—

A carcass of beef with 12 stamps.
A carcass of veal with 6 stamps.
A carcass of mutton with 8 stamps.
A carcass of pork with 12 stamps.

Persons requiring more marks put on the meat than those stated must make application at the time the stamping is being done, and point out where the additional marks are to be placed.

Regulations as to the Freibank

This is an establishment to which all meat which is passed as fit for food, but which is of an inferior quality, is taken, in order that such meat, being of less value than meat which is fully nourished, shall, under the supervision of the authorities, be sold as meat of an inferior quality, and at prices to be fixed by them, which must not exceed four-fifths of the market prices of good meat.

All fresh meat which has, upon examination by the veterinary surgeon, been found to be of an inferior quality, shall be taken to the Freibank; such meat can only be sold at the Freibank and by persons employed by the City Council.

Such persons shall not sell any other kind of meat, nor shall they sell meat outside the Freibank.

The Council shall fix the locality and time for the holding of the Freibank.

The flesh of animals slightly diseased, which has been thoroughly boiled on the premises, may be sold at the Freibank, but it must be stated at the time of sale what the animal has been affected with.

The meat shall be weighed to the salesman, who shall account for all meat given him to sell.

The meat shall be sold in pieces not exceeding 6 lbs. in weight, and for private consumption only.

The amount realized from the sale of meat at the Freibank shall, after deducting expenses and fees, be handed over to the owners of the meat.

Compensation for Closure of Private Slaughterhouses

In Germany compensation is paid for closing the private slaughterhouses. The method of determining the amount being by arbitration; the owner of the slaughterhouse appoints one arbitrator, the Town Council another, and the Provincial Government a third. In any case the owners have to give notice of their intention to claim compensation within six months after they have received notice to close, or no claim will be considered.

This question of compensation, you may remember, was not touched on by the Royal Commission on Tuberculosis in their recommendations. It was simply suggested that three years' notice should be given. It is, of course, an important point; and it is here again that the help of the family practitioner can be of service in rousing interest in the matter, and so gradually forming public opinion.

In considering the question of compensation two classes or persons concerned have to be mentioned, one of which classes may be again subdivided.

1. The people whose slaughterhouses are unsatisfactory and insanitary in every way. Are such people to be compensated for being compelled to desist from perpetuating a most unsatisfactory condition of things? I think not.

2. There are owners and tenants of slaughterhouses who do everything they can, under the circumstances, to carry on their business with as much regard as possible to the public health. They keep their premises in good order, and are always ready, as far as possible, to modernize them to meet the suggestions of the medical officer of health. These people deserve better treatment in the way of compensation or length of notice than the first class I mentioned.

It seems to me, however, that the question of compensation need not be so large a one as we have been led to suppose, for there is little necessity to compensate any but those whose actual business as slaughterers is stopped.

The better class slaughterhouse owner that I have been speaking of is either: (*a*) A butcher who uses the private slaughterhouse for his own convenience, for example, as being convenient to his shop, or for other reasons; or (*b*) a slaughterer who is not a meat seller at all, and simply lives by his business of slaughtering for others.

Surely, if it is in the interests of the public health that private slaughterhouses should be closed, the mere question of convenience to the butcher and meat seller should not entitle him to compensation for disturbance. He would have his three years' notice, and at the end of that time let him do his slaughtering at the proper place, namely, the public abattoir; and if, in any special case, it were shown that he had perhaps recently spent considerable sums of money in endeavouring to comply with the requirements of the medical officer of health of the district, some basis of compensation might be easily arrived at.

But with the other man the case is somewhat different. He has a trade on which he lives, and at the end of three years that source of livelihood comes to an end. Should it be a very serious matter to compensate him? and if some compensation is necessary, could not it take the form of guaranteed employment at the new abattoir, if he is a working man? If, on the other hand, he did not himself actually use the poleaxe, he could receive an annuity or lump sum, calculated on a fair and moderate basis.

The German Act of 1881 specially provides that no compensation shall be paid for the extra trouble there may be involved by reason of a butcher having to slaughter away from his own place of business. Compensation paid shall be for real damage or loss which can be proved on account of the closing of the places; such places having been built for slaughtering purposes. And compensation to be paid must have deducted from it the amount which may be realized from the properties and fixtures when used for other purposes.

I think, in England, the difficulties have been exaggerated. Let public opinion be sufficiently aroused on the subject, and I do not think the question of compensation will be a very serious difficulty. At present public opinion is not strong; on the other hand the butchers and their federations are, and, naturally, the Government says 'the time is not yet ripe.'

Stamping Meat

With regard to the question of stamping the meat it has been suggested that the British public will object to have its joints of meat disfigured thereby. I do not believe so for one instant. In Germany such marks are understood, looked for, and sometimes even more of them asked for on a carcass than the authorities usually put; and why should it not be the same in England?

THE DISINFECTION OF THE EXCRETA.*

By CHARLES A. HILL, B.A., M.B., B.C. Cantab.

AND

JOHN HILL ABRAM, M.D., Lond., M.R.C.P., Assistant Physician Royal Infirmary, Senior Demonstrator in Pathology, University College Liverpool

From the Bacteriological Laboratories, University College Liverpool. Read at a meeting of the Lancashire and Cheshire Branch.

The methods used for ascertaining the value of any disinfectant are mainly two, namely, the addition of definite quantities of the substance to an active broth culture, with subsequent inoculation upon various media after the lapse of varying intervals, and the action of solutions of known strength upon test organisms inoculated upon threads.

These methods are valuable in that they determine the absolute working power of the disinfectant in definite conditions, but we have felt that direct experiment is necessary before conclusions derived from them can be adopted. This is more especially to be borne in mind in connection with the subject of our communication.

We have limited our inquiry to the disinfection of the fæces, as, naturally, they are the most resistant. In our earlier experiments the disinfectant solution was added in excess to the solid fæçal mass, but we speedily found that in not a single instance did we get any sterilization of the central portions. Therefore we would insist upon the necessity for thoroughly mixing up the fæcal matter with the disinfectant selected.

We have chosen half an hour as the time during which the mixture must be allowed to stand as being a convenient one in practice, though we admit that it is a severe test, and no doubt explains some of our results. After exposure for half an hour, plate cultivations were made in the usual manner with gelatine and agar-agar.

---

* Reprinted from the 'British Medical Journal,' April 16th, 1898.

The accompanying tables give our results at a glance. The second table gives the minimum strength of those disinfectants which seem to act most satisfactorily. The price list is only approximately correct :—

TABLE I

Disinfectant.	Strength.	Action on fæces.	
		When mixed.	When unmixed.
Perchloride of mercury	1 in 1,000	Spore-bearing forms	Copious growth
Formol	1 in 20	Sterile	Copious
Carbolic acid ...	1 in 20	Sterile	Copious
Iodic hydrarg. ...	1 in 4,000	Spore-bearing forms	Copious
Chloros	Undiluted	Limited	Limited
Chinosol	1 in 600	Sterile	Copious
Izal	1 in 200	Spore-bearing forms	—
Creolin	1 in 10	Sterile	
Carbolic acid (crude)	Undiluted	Sterile	—
Zinc chloride ...	1 in 10	Copious growth	—
Copper sulphate ...	1 in 20	Copious	—
Boiling water ...	—	Copious	—
Sodium chloride ...	1 in 10	Copious	—
Chloride of lime, 15% Cl.	1 in 10	Copious	—
„ „ 35% Cl.	1 in 10	Considerable	—

TABLE II

MINIMUM STRENGTH OF SIX SOLUTIONS WITH APPROXIMATE COST PER GALLON

Carbolic acid	1 in 20	... ...	1s.
Crude carbolic	1 in 40	... ...	6d.
Formol	1 in 40	... ...	1s. 6d.
Chinosol	1 in 600	... ...	1s.
Creolin	1 in 40	... ...	1s. 6d.
Mercuric chloride ...	1 in 500	... ...	9d.

The above six solutions were tested with a typhoid stool, and all gave complete sterilization.

The literature on the subject is small, at any rate from our special standpoint. UFFELMANN* states that the mineral acids are the most active of all disinfectants, then corrosive sublimate, carbolic acid, etc. He points out the importance of the duration of the influence of the disinfectant upon the motions. He recommends sulphuric or hydrochloric acid diluted with double the quantity of water, mixed in equal bulk with the fæces, and allowed to stand, in the case of the former acid, 2 hours, of the latter 12 hours. FOOTE† comes to the following conclusions—that the best disinfectants to use are corrosive sublimate with hydrochloric acid, corrosive sublimate with potassium permanganate, and the chloride of lime. He further states that

* Uffelmann : 'Die Desinfection infectiöser Darmentleerungen,' 'Berl. klin. Woch.,' 1889, No. 25.

† Foote : 'The Sterilization of Fæces.' 'Intern. Jour. Med. Sciences,' Oct. 1889.

solutions of chloride of lime should be kept tightly corked, and should not be used after they are a week old. GILMAN THOMPSON* recommends 1 in 500 perchloride, 1 in 10 crude carbolic and chlorinated lime. He states that the mass must be thoroughly mixed and allowed to stand two hours. These workers recommend mineral acids, corrosive sublimate, crude carbolic, and chlorinated lime. It will be seen from the table that we have found corrosive sublimate and crude carbolic good disinfectants. The mineral acids we have not tested, as there are many and obvious objections to their use. Chlorinated lime we find with our time limit of half an hour to be useless. It may be said the preparations were old, but we submit that such an objection necessarily puts the substance out of court. Any of the six disinfectants on our select list may be regarded as absolutely certain in their results when thoroughly mixed with the stool and allowed to stand for half an hour. There are, however, objections to the use of some of them.

Corrosive sublimate is a scheduled poison, acts upon the metal work of the drainage system. and, moreover, gives a red colouration with stercobilin. This latter point is of importance, inasmuch as it may mask the presence of blood in a typhoid stool; again, albumen is coagulated by mercuric chloride, and thereby protects organisms enclosed in the centre of the coagulum. Crude carbolic acid is cheap and efficient, but does not mix over well with fæces; it stains linen, and is poisonous. Formol and creolin are good, but are rather costly. The remaining substance, chinosol, is, in our opinion, the best. It is reliable, an excellent deodorant, and mixes well with the fæces. It is dearer than crude carbolic, but its cost is more than counterbalanced by its portability. It is put on the market in tablet form. The tablets are readily soluble, and may be obtained in such strength that one of them in a pint of water makes a solution of effective strength.

We may sum up our paper with the following conclusions:—

1. It is absolutely necessary to mix the fæces thoroughly with the disinfectant.
2. The mixture should stand at least half an hour.
3. Carbolic acid, crude carbolic acid, formol, creolin, chinosol, and corrosive sublimate in the strengths given in the short list are all effective, but chinosol seems the most convenient.

---

* Gilman Thompson: 'Albany Med. Annals,' March and April, 1897.

BORIC ACID AND FORMALIN AS MILK PRESERVATIVES*

By H. E. ANNETT, M.D., D.P.H. Vict.
Thompson Yates Laboratories, University College Liverpool

'There is no doubt that boric acid, if taken in large quantities, would be injurious to health, but we have not sufficient information to show whether such minute quantities as are generally added as preservatives would be regarded as having that effect; and more exact information is wanted before it can be decided whether a process which, *primâ facie*, may be regarded as intended to prevent the loss of valuable foods must be held to be prohibited by law.' These were the words in which, in 1890-91, the Committee of Inquiry of the Local Government Board gave its opinion on the important question of the use of preservatives in foods. It is remarkable that 'sufficient information' on the action of minute doses of 'preservatives' habitually taken, after the lapse of a period of almost ten years, is still not forthcoming. The most recent legislation indicates the absence of such information. Thus Article 1, section 7, of the Sale of Foods and Drugs Acts, 1899, states: 'Provided that an article of food shall not be deemed to be adulterated by reason only of the addition of any preservative or colouring matter of such a nature and in such quantity as not to render the article injurious to health.' The 'Lancet,' at the time of the appointment of the Adulteration of Foods, &c., Commission in 1896, urged the importance of an inquiry into the question of preservatives, and by means of the '"Lancet" Sanitary Commission on the Use of Antiseptics in Food,' published the opinions of the most eminent authorities of this country. These were unanimously of the opinion that the use of antiseptics in food was injurious to health; but they could produce no direct evidence in support of their opinions. The Local Government Board have recently appointed a special committee 'to inquire into the use of preservatives and colouring matters in food, and to report (1) whether the use of such materials, or any of them, for the preservation and colouring of food in certain quantities is injurious to health, and if so, in what proportions does their use become injurious; (2) to what extent and in what amounts are they so used at the present time.'

The Use of Boric Acid and Borax

Boric acid was introduced into medicine in 1702 by Homberg, as a sedative salt with anodyne and anti-spasmodic properties. In 1844, Binswanger, in the Münich Prize Essay, considered boric acid to be comparatively inert in its action, doses of from 1 to 3 drachms causing gastro-intestinal irritation; borax also caused similar symptoms and often an impetiginous

* Reprinted from the 'Lancet,' November 11, 1899.

eruption. WILD,* reviewing a larger number of cases which he had collected from English and foreign literature, sums up the question of boric acid and borax intoxications thus: 'Two forms are recognized—1. In which large quantities of the drugs are rapidly absorbed from the alimentary canal from serous and other cavities, or from extensive raw surfaces; in these cases vomiting, diarrhœa, general depression, and partial paralysis of the nervous and muscular systems occur and may cause death. Erythematous and impetiginous rashes are common. 2. In which the drug is administered in small doses for long periods. Whether the condition of the kidneys, or an individual idiosyncrasy in regard to the drug, is the determining factor in causing toxic symptoms requires further investigation; but it is an important fact that the great majority of persons taking boric acid or borax do so without injurious effects. GOWERS,† LIVEING, FERE,‡ and others recorded that, in several epileptic cases undergoing a course of treatment with boric acid or borax for a long period, in some cases there were considerable gastro-intestinal irritation, nausea, and vomiting; they also noted the occurrence of a dry condition of the skin and mucous membranes, falling out of hair, striation of the nails, psoriasis, erythema, a special papular eruption, desquamation, petechiæ, œdema of the extremities, albuminuria, debility, and anæmia. The doses in these cases amounted to from 4 to 10 grammes a day (about from 60 to 160 grains). WILD and HALL§ record also several cases with similar symptoms which had been treated with doses of boric acid, or a mixture of that acid and borax, amounting only to from 30 to 75 grains daily. Doses of 80 grains per day for a single day produced no effect on 40 of WILD's cases; but doses amounting to 120 grains in 4 hours produced in WILD himself nausea, colic, diarrhœa, headache, depression, loss of appetite, and flushing of the skin. The only direct evidence of injurious effects being produced by the ingestion of 'preserved milk' is obtained from the cases recorded by Dr. M. K. ROBINSON,‖ the Medical Officer of Health of the East Kent Combined Sanitary District. Five of the seven inmates of a certain house became suddenly ill after partaking of blanc-mange which had been made from milk of the previous day, to which, as was confessed, the dairyman supplying it had already added boric acid, and to which the cook had added a further quantity to preserve it over-night. Nine fowls fed liberally with the blanc-mange became ill, five dying.

EXTENT OF THE USE OF BORIC ACID, &c.

Dr. A. HILL's¶ paper, read before the Society of Medical Officers of Health, furnishes valuable statistics from Birmingham. Out of 2,300 samples of food, 460, or 20 per cent., contained either boric acid, formol, or salicylic acid. Boric acid was found in 35 per cent. of 882 samples of butter and margarine, and in 15 or 24 samples of bacon, sausage, and other animal foods. In 5 per cent. of 1,360 samples of milk boric acid was contained in quantities varying from 3 to 130 grains per gallon. The samples of butter analyzed contained boric acid varying in quantities from 7 to 84 grains per pound, or from 0.1 per cent. to 1.2 per cent. Five out of eight samples of cream

---

* The 'Lancet,' Jan. 7th, 1899, p. 23.
† *Ibid.*, Sept. 24th, 1881, p. 546.
‡ *Ibid.*, Oct. 12th, 1895, p. 932.
§ The 'Lancet,' Jan. 28th, 1899, p. 261.
‖ 'Public Health,' August, 1899.
¶ *Ibid.*, May, 1899.

contained boric acid. Several prosecutions in different towns of England are recorded of cases in which similar quantities of boric acid have been used.

Action of Boric Acid

Professor Allen,* Professor of Physiology in Mason College, Birmingham, stated :— 'Boric acid and its compounds are quite foreign to the animal body, and their presence must necessitate departure from the normal chemistry of life. The departure may be expected to consist partly in the formation of inert compounds of boric acid with calcium and magnesium (perhaps also iron), and the consequent removal of these indispensable metals from the economy. Whatever antiseptic may be used it should be remembered that, in order to obtain its due effect, there must be enough present to exert a decided influence on protoplasmic activity. This effect can hardly be expected to be beneficial to the tissues of the alimentary canal. Even the disinfection of the contents of the canal is a doubtful blessing in most cases, because the beneficial organisms are thereby destroyed. However harmless an occasional dose of boric acid (or one of its compounds) may be, it is evident that the case may be different when nearly every article of diet contains it.'

The most systematic and complete experiments as to the action of boric acid and borax on the digestive ferments are those of Rideal and Foulerton.† They used a mixture of boric acid (25 per cent.) and borax (75 per cent.). The following are abstracts of their results :—

A.—Action on Amylolytic Digestion

1.—*Salivary Digestion of Starch.*

Preservative.	Strength.	Retarding effect of preservative.
Boric mixture - -	1 in 2000	11.7 per cent.
,,	1 ,, 1000	12.2 ,,
,,	1 ,, 333	14.0 ,,

The retarding effect was measured by the amount of sugar formed as compared with a control sample containing no preservative.

2.—*Digestion of Starch.*

Preservative.	Amount.	Retarding effect on action of		
		Zymine.	Pancreatine.	Liquid Taka-diastase.
Boric acid mixture	1 in 2000	22.8 per cent.	36.0 per cent.	67 per cent.
,, ,,	1 ,, 1000	37.0 ,,	46.4 ,,	73 ,,
,, ,,	1 ,, 333	49.2 ,,	53.0 ,,	80 ,,

* Report of the 'Lancet' Sanitary Commission on the Use of Antiseptic in Food, the 'Lancet,' Jan. 2nd, 1897, p. 58.
† 'Public Health,' May, 1899.

B.—Proteolytic Digestion

1.—Pepsin digestion of raw beefsteak.

Preservative.	Amount.	Method of experiment.	N. dissolved.
Nil.	—	Digested for one hour.	46.8 per cent.
Boric acid mixture.	1 in 2000	Preservative and steak in contact for 24 hours before adding pepsin and acid.	45.5 „
„	1 „ 1000	„ „	42.2 „
„	1 „ 333	„ „	34.6 „
„	1 „ 1000	Digested for one hour.	50.7 „

2.—Pancreatic digestion.

Preservative.	Amount.	Method of experiment.	Retarding action.
Boric acid mixture.	1 in 2000	Preservative in contact 20 hours before digestion.	0.6 per cent.
„	1 „ 1000	„ „	2.7 „
„	1 „ 333	„ „	2.4 „
„	1 „ 1000	Without previous contact.	1.0 „

C.—Influence of Curdling of Milk by Chymosin (Rennet Ferment)

Very little influence was noted except that the borated samples showed more tendency to separate and that the curd was slightly more solid.

Several other investigators—Cripps,* Liffman,† and others—have obtained results differing in a very few details from these. The figures show that boric acid and borax have a detrimental action on all the digestive ferments to the extent of from 0.6 to 53 per cent., according to the amount used and the nature of the ferment action.

Experiments on Animals

Neumann‡ recorded the earliest experiments on animals. Dogs (in weight about 15 kilogrammes) received from 5 to 6 grammes of boric acid without effect beyond a fall of temperature. Larger doses produced vomiting and diarrhœa. Doses of 10 grammes and over produced death by nerve and muscle paralysis. Rabbits, Pigs, Horses, and Fowls gave

* The 'Analyst,' 1897, p. 184. † *Ibid.*, p. 102. ‡ 'Archiv für Experimentelle Pathologie und Pharmacologie,' 1881.

similar results. CHITTENDER and GIES* experimented with full-grown Dogs (in weight from 8 to 12 kilogrammes) which were dosed during a period of from 27 to 46 days with borax and boric acid. They found that moderate doses of borax, up to 5 grammes daily, were without influence on metabolism or on general nutrition while in doses of from 5 to 10 grammes daily borax disturbs metabolism, increasing excretion of nitrogen and of sulphuric and phosphoric acids through the urine. Boric acid up to 3 grammes had no effect. Still larger doses of borax or of boric acid disturbed the assimilation of proteids and fats, increasing the amount of fæces and of their nitrogen and fat. Diarrhœa and an increase of mucous secretion were also produced. Thus all experimental work on the subject has been performed on adult animals. No records of experiments with young animals have, as far as I can ascertain, been published. Many have stated that the quantities of boric acid used as preservatives are injurious to infants, without producing direct evidence to that effect. It has been often pointed out that the average quantities present in 'preserved' milks is more than the ordinary maximum British Pharmacopœia dose for an infant taking from 32 to 40 ounces of milk per diem.

Most authors† are agreed that not less than 35 grains per gallon (0.05 per cent.) of a mixture of boric acid and borax is a minimum efficient quantity necessary to 'preserve' milk for 48 hours, while this quantity is rather more than sufficient for a period of 24 hours. In order to obtain some better indications of the probable action of boric acid on young infants and children I arranged a number of experiments on young kittens, 3 or 4 weeks old, just able to lap milk. Their condition at this age seemed more analogous to that of infants than in the case of most other animals, as they are entirely dependent on milk for their food until they reach the age of about 2 months. The experiments were made with boric acid—quantities of 40 grains and 80 grains per gallon being employed. Judging from the experiments of CHITTENDER and GIES on adult Dogs, boric acid seems to have a less deleterious effect than borax. The experiments were only preliminary, and a further series, with less quantities of acid, are in progress. The animals were kept under the most favourable circumstances, being allowed during the greater part of the day to run about within a considerable area, and at night they were housed in capacious cages in the animal house at the laboratory. A supply of fresh milk, renewed every few hours during the day, was always provided. As the animals grew older a little bread was added, and later a little meat was also given. The results were very striking, as the following tables show.

* 'American Journal of Physiology,' vol. i, 1898, No. 1. † Rideal and Foulerton : 'Public Health,' May, 1899, p. 534.

A.—Five Kittens Treated with Milk Preserved by Boric Acid in Quantity of 80 Grains to the Gallon

No.	Original weight.	Weight at end of			
		First week.	Second week.	Third week.	Fourth week.
1	388 grammes	410 grammes	387 grammes	313 grammes	Dead.
2	497 „	490 „	500 „	423 „	„
3	468 „	402 „	385 „	328 „	„
4	468 „	472 „	420 „	438 „	„
5	342 „	327 „	331 „	332 „	„

B.—Kittens Dosed with Milk Containing 40 Grains to the Gallon

No.	Original weight.	Weight at end of			
		First week.	Second week.	Third week.	Fourth week.
1	442 grammes	395 grammes	340 grammes	330 grammes	Dead.
2	345 „	335 „	308 „	Dead.	—
3	268 „	253 „	215 „	„	—
4	540 „	482 „	488 „	500 grammes	Dead.
5	495 „	462 „	440 „	502 „	„

C.—Kittens Receiving Untreated Milk

No.	Orlginal weight.	Weight at end of				
		First week.	Second week.	Third week.	Fourth week.	Fifth week.
1	457 grammes	486 grs.	443 grs.	500 grs.	492 grs.	500 grs.
2	354 „	446 „	452 „	515 „	505 „	603 „
3	485 „	562 „	686 „	732 „	865 „	853 „
4	264 „	265 „	300 „	362 „	—*	—
5	313 „	320 „	333 „	340 „	384 „	441 „

It was endeavoured to have kittens of as nearly as possible about the same weight and of the same age and born of the same Cat in each of the groups A, B, and C, so that the conditions might be approximately the same. The earliest symptom manifested by the kittens of Groups A and B was loss of appetite; it was seen, even after the first day or two, that they took less milk than the control kittens. Diarrhœa, inactivity, and depression soon followed, then rapid emaciation and death. It is very evident from these experiments that much smaller quantities than 40 grains

* Strayed and lost.

to the gallon would have considerable effect, and experiments to test this are in progress. The results, I think, of these experiments cannot fail to lead us to infer that similar if not more injurious effects would be produced by repeated administration of similarly 'preserved' milk as the daily food of young infants, and all the evidence tends to prove that milk, especially during the summer months, is habitually and systematically 'doctored.'

The Use of Formalin

Formalin has only been introduced commercially within the last two or three years. It has been but little used in medicine except as a diluted vapour in the treatment of phthisis and whooping-cough, and no exact dosage has been assigned to it. Formol, formal, and formalin are terms in use for the commercial liquid which contains about 40 per cent. of pure formaldehyde (CHOH). The extent of its use as a milk preservative is seen from Dr. Hill's figures. Dr. Hill found formalin in 47 out of 1,000 samples of milk examined. In some American cities its use had become so extensive that the sanitary authorities* provided each milk inspector with a small chemical outfit, consisting of ferric chloride solution, sulphuric acid, pipettes, and test-tubes, in order to test for formalin. After the passing of an act in 1895 prohibiting the addition of any preservative to milk the open use of formalin ceased, but substitutes were presented for patronage under various names, such as 'freezine,' 'milk sweet,' 'callerine,' &c., which were found to be preparations of formalin. Costly circulars, booklets, and pamphlets were largely distributed to milkmen extolling the merits of the various preparations. The use of these was often described as 'a new process for keeping milk and cream sweet *without ice*.' The user was assured by the circulars that 'it cannot be detected when used—in fact, a chemist could not find any trace of it if the milk were analyzed.' The medical officer of health of Philadelphia describes its use 'as wholly mercenary, regardless of the injurious effects upon the digestion, especially of children and invalids.' Cassal† gives his opinion that 'formic aldehyde is a substance which must be regarded as even more objectionable than boric acid.'

Action of Formaldehyde on Digestive Ferments

Rideal and Foulerton extended their experiments also to the action of formalin with the following abstracted results :—

A.—Amylolytic Digestion

1.—Salivary Digestion of Starch.

Strength of formalin.	Retarding effect.
1 in 100,000	0.2 per cent.
1 „ 50,000	4.0 „
1 „ 10,000	11.0 „

* Annual Report of the Bureau of Health, Philadelphia, 1898.

† 'Public Health,' 1899, p. 535.

2.—Digestion of Starch.

Strength of formalin.	Retarding action on		
	Zymine.	Pancreatine.	Liquid Taka-diastase.
1 in 100,000	3.6 per cent.	13.0 per cent.	5.0 per cent.
1 „ 50,000	8.2 „	16.0 „	8.5 „
1 „ 10,000	8.5 „	16.7 „	18.0 „

B.—Proteolytic Digestion

1.—Pepsin Digestion of Raw Beefsteak.

Strength.	Retarding Action.
1 in 50,000	2.6 per cent.
1 „ 100,000, after 24 hours' previous contact.	8.6 „
1 „ 50,000 „ „	8.7 „
1 „ 10,000 „ „	12.6 „

2.—Pancreatic Digestion: Digestion of Casein.

Strength.	Retarding Action.
1 in 50,000	0.3 per cent.
1 „ 100,000, after 20 hours' previous contact with preservative.	5.4 „
1 „ 50,000 „ „ „	5.9 „
1 „ 10,000 „ „ „	8.6 „

Thus formaldehyde, in strengths of from 1 in 50,000 to 1 in 10,000, appears to have a deleterious effect on the digestive ferments to an extent varying from 0.2 per cent. up to 18 per cent., so that it is possible that the total effect of formalin, after acting on the different digestive ferments on its way through the alimentary tract, might amount to a very considerable total. Rideal and Foulerton,* from experiments to ascertain the 'preserving' effect of formalin on milk from a bacteriological point of view, decided that not less than 1 part in 50,000 is necessary to preserve milk for 48 hours. There is but little doubt that milk, after passing through the hands of the wholesale purveyor, dairyman, and milkman, often contains considerably more than this quantity.

* *Loc. cit.*

Experiments on Animals

The only recorded experiments are those of Rideal and Foulerton on 3 Cats, 1 Rabbit, and 2 Guinea-pigs. The Cats, which were 3 months old, and the Guinea-pigs, which were over 200 grammes in weight, were not in such a condition as to furnish the best indication of the action of formalin. No effect was noticed after a course extending over some weeks. I therefore arranged a large number of experiments on young kittens, 3 or 4 weeks old, kept under conditions similar to those in the experiments with boric acid. The results are noted in the following tables.

A.—Kittens Receiving Formaldehyde 1 Part in 50,000 of Milk

No.	Original weight.	Weight at end of						
		First week.	Second week.	Third week.	Fourth week.	Fifth week.	Sixth week.	Seventh week.
	Grms.	Grms.	Grms.	Grms.	Grms.	Grms.	Grms.	Grms.
1	273	271	305	336	399	—*	—	—
2	279	275	285	326	370	434	463	—
3	465	447	409	—*	—	—	—	—
4	425	420	445	438	410	537	646	590
5	315	303	290	367	331	—*	—	—

Control Kittens Receiving Untreated Milk

No.	Original weight.	Weight at end of					
		First week.	Second week.	Third week.	Fourth week.	Fifth week.	Sixth week.
	Grms.	Grms.	Grms.	Grms.	Grms.	Grms.	Grms.
6	315	320	333	340	384	441	—
7	425	486	450	583	680	744	836
8	430	380	358	445	547	609	—
9	432	473	478	538	672	731	—

B.—Kittens Receiving Formaldehyde 1 Part in 25,000 of Milk

No.	Original weight.	Weight at end of					
		First week.	Second week.	Third week.	Fourth week.	Fifth week.	Sixth week.
	Grms.	Grms.	Grms.	Grms.	Grms.	Grms.	Grms.
1	408	416	518	510	537	558	583
2	405	485	568	567	612	630	585
3	539	565	717	692	706	700	713
4	537	575	695	727	764	760	793

* Dead.

Control Kittens Receiving Untreated Milk

No.	Original weight.	Weight at end of					
		First week.	Second week.	Third week.	Fourth week.	Fifth week.	Sixth week.
	Grms.	Grms.	Grms.	Grms.	Grms.	Grms.	Grms.
5	544	615	756	830	955	900	883
6	424	509	633	627	682	635	639
7	485	562	686	732	865	853	868

C.—Kittens Receiving Formaldehyde 1 Part in 12,500 of Milk

No.	Original weight.	Weight at end of					
		First week.	Second week.	Third week.	Fourth week.	Fifth week.	Sixth week,
	Grms.	Grms.	Grms.	Grms.	Grms.	Grms.	Grms.
1	483	535	543	465	—*	—	—
2	470	498	505	468	—*	—	—
3	550	556	566	525	445	487	514
4	389	435	470	485	540	580	—
5	395	365	401	462	497	581	—

Control Kittens Receiving no Formaldehyde

No.	Original weight.	Weight at end of					
		First week.	Second week.	Third week.	Fourth week.	Fifth week.	Sixth week.
	Grms.	Grms.	Grms.	Grms.	Grms.	Grms.	Grms.
6	544	615	756	830	955	900	—
7	354	446	452	515	505	603	726
8	408	385	433	538	672	731	—

The following tables show the average weekly increase :—

Kittens A.		Grms.
1st week, average of 5 kittens ...	−	8.2
2nd „ „ „ „ ...	+	3.6
3rd „ „ „ „ ...	+	28.4
4th „ „ 4 „ ...	+	8.6
5th „ „ 2 „ ...	+	145.2
Average total increase for 5 weeks		177.6

Control Kittens.		Grms.
Average of 4 kittens	+	14.7
„ „ „	−	10.0
„ „ „	+	89.2
„ „ „	+	96.7
„ „ „	+	60.5
		251.1

* Dead.

Kittens B.		Grms.
1st week, average of 4 kittens ...	+	38.0
2nd " " " " ...	+	114.2
3rd " " " " ...	−	0.5
4th " " " " ...	+	30.5
5th " " " " ...	+	7.2
6th " " " " ...	+	7.2
Average total increase for 6 weeks		196.6

Control Kittens.		Grms.
Average of 3 kittens	+	77.6
" " "	+	129.6
" " "	+	61.3
" " "	+	104.6
" " "	−	38.0
" " "	+	0.6
		325.7

Kittens C.		Grms.
1st week, average of 5 kittens ...	+	20.4
2nd " " " " ...	+	25.4
3rd " " " " ...	−	18.0
4th " " 3 " ...	+	13.3
5th " " " " ...	+	55.3
Average total increase for 5 weeks		96.4

Control Kittens.	Grms.
Average of 3 kittens	46.6
" " "	68.3
" " "	80.6
" " "	83.0
" " "	34.0
	312.5

The earliest effect of the formaldehyde was to cause a loss of appetite during the first week. Diarrhœa with noisy gaseous motions, gaseous distension of the abdomen, and changes in the fur (the hairs standing and feeling rough and unpleasant to the touch) followed. In some cases emaciation and death occurred; in others the condition continued with but little increase in weight until the animals reached the age of about two months. The younger the animals the more susceptible they proved to the action of formalin. Compared with the control animals it is seen that the formalin in Group A (1 in 50,000) retarded the nutrition of the animals, as indicated by their increase in weight, to the extent of about 29.3 per cent., in Group B (1 in 25,000) to 39.6 per cent., and in Group C (1 in 12,500) to 69.1 per cent. It is evident, from a study of the figures, that experiments should be continued on a larger number of animals to avoid the very material effect which one animal in five may cause on the average.

From a consideration of the results of the above tests—chemically of the effect of boric acid and formalin on the different digestive ferments, and physiologically of the effect of these re-agents on the nutrition of young kittens—one is forced to conclude that these chemicals when used as preservatives of milk (and probably of other foods) are very injurious to the health of the consumer and particularly so to the health of young infants. Further, it is easy to conceive that the great infant mortality rate from diarrhœa of many of our large towns may be closely connected with the practice, especially during the summer months, of systematically 'doctoring' milk by means of the preservatives used by milk purveyors, dairymen, and milkmen. These experiments on young animals will constitute the first of a large series, by which that 'sufficient information' as to the injurious effects of the use of preservatives in milk will be provided, in order to make an action under the Sale of Foods and Drugs Act possible.

CARCINOMA OF THE KIDNEY ARISING IN THE GLOMERULI*

By JOHN HILL ABRAM, M.D. Lond., M.R.C.P. Assistant Lecturer on Pathology University College, Assistant Physician, Royal Infirmary Liverpool

From the Thompson Yates Laboratories, University College Liverpool.

My object in reporting this case is to place on record what seems to be an almost unique condition in the kidneys.

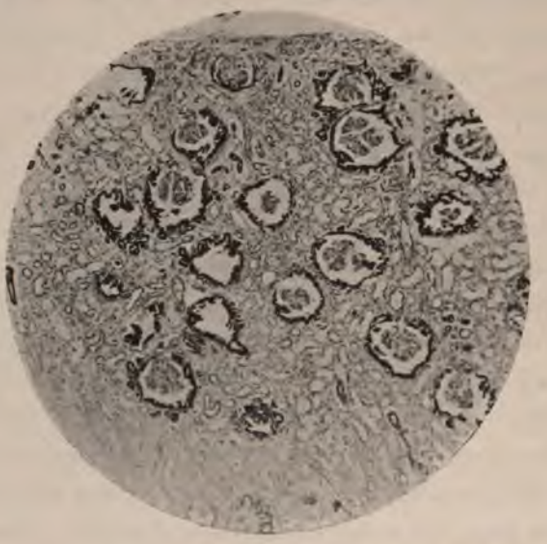
Clinical History.—William D., æt. 16, a groom, was sent to the Royal Infirmary by Dr. Blair Bell, of New Brighton, and was admitted under my care. He gave an eight months' history of pain in the back. He was emaciated and anæmic. Enlarged glands in groins, axilla, and submaxillary regions. Two tumours on the anterior surface of the sternum, over these the superficial veins distended. Liver enlarged. Urine (by catheter) 1025, no sugar, no albumin. Paresis in legs, knee-jerks present. Incontinence of urine and fæces. Fresh tumours developed on the ribs; the liver became larger and nodular; the weakness in the legs more marked; the knee-jerks no longer obtainable. Towards the end, cough, blood-stained sputum; thrombosis in left iliac vein; left hydrothorax. Death after ten months' illness.

Post-mortem.—Numerous growths on ribs. Two masses of growth on the anterior surface of the sternum, a large mass on the posterior surface. For 3 in. above and below the sixth dorsal vertebra extensive growth in the pre-vertebral connective tissue, also infiltrating the nerve roots. Some patches of growth on the outer surface of the dura mater spinalis. The pleural cavities contain clear fluid. Both layers of pleuræ and the lungs show numerous nodules of new growth. Liver enlarged, right lobe practically replaced by growth, many nodules in left lobe. Spleen normal. Kidneys rather large, one or two small nodules of growth. The growth everywhere is firm and white.

Histological Examination.—Portions of the various organs were hardened in the usual fluids, and the histological examination revealed a very interesting although unexpected condition. The new growth on the sternum was found to be not a connective tissue tumour, but epithelial in nature. The tumour tissue contained somewhat slender cells packed in irregular alveoli, the central portions showed many necrotic cells, and in parts the stroma had undergone myxomatous degeneration. The growth was limited to the periosteal surface of the bone, and the medullary substance was normal. The nodules on the dura mater presented a similar structure; the nerve roots contained many degenerated nerve-fibres. The cord itself was practically normal. The growth in the liver resembled that on the bones. Here, too, the myxomatous change in the

* Reprinted from the 'Journal of Pathology and Bacteriology,' February, 1900.

centre of the nodules was marked. The epithelial masses in the periphery partly infiltrated, partly compressed the surrounding liver substance. The kidneys, in addition to a slight patchy sclerosis of the cortex, presented a most unusual appearance. Throughout the cortex the glomeruli were striking objects, as is well seen in the figure. The prominence of the glomeruli was due to deeply-stained epithelium lining the parietal layer of BOWMAN's capsule. A further examination showed the epithelial lining to consist of several layers of slender columnar cells with oval nuclei. A marked feature in the majority of the glomeruli was the normal appearance of the vascular tuft and its epithelial covering. In a few instances the proliferating epithelium completely filled the capsule. In places the growth penetrated the basement membrane and infiltrated the surrounding tissue, and in not a few cases the epithelium of the renal tubules in immediate relationship to the glomeruli was replaced by that of the growth. The small nodules of white growth were found to consist of masses of cells with a considerable amount of stroma, in which some bundles of fibres resembling muscle-fibres were present.



The general appearance of the glomeruli is well seen. The dark layer consists of several layers of slender columnar cells; a similar deeply-stained epithelium is present in the tubules adjoining some of the glomeruli. The normal appearance of the epithelial covering of the vascular tuft is obvious.

The occurrence of a malignant epithelial tumour at the age of 16 years is very unusual, and the condition of the kidney is almost unique. I have been able to find only two cases on record which in any way resemble my own.

REMARKS

O. HILDEBRAND* reports a case of small cell carcinoma in a child æt. 5. The *referat* says HILDEBRAND considered the tumour to arise in the glomeruli, and that the proliferating epithelium therein finally infiltrated the surrounding tissue.

---

*'Arch. f. klin. Chir.,' Berlin, 1894, Bd. xlviii. Refer. 'Centralbl. f. allg. Path. u. path. Anat.,' Jena, 1895, Bd. vi, S. 519.

A case bearing a very close resemblance to mine was reported by SEYMOUR SHARKEY* in 1882.

G. R., married woman, æt. 28, emaciated, anæmic, jaundiced ; liver enlarged ; tumour above pubes.

Post-mortem.—Nodules of new growth in lungs. Liver : masses of new growth. Right ovary cystic, new growth in remaining portion. Kidneys *appear normal.*

Histology of Kidney.—Glomeruli : the basement membrane of the capsules is sharply defined, and the capillaries within are healthy, but from the internal surface of the basement membrane rose a thick deeply-stained wall of cells, having a very clear columnar arrangement. In some of the larger areas of disease the earliest changes were in the epithelium of the tubules, which was transformed into a columnar epithelium.

I have given a somewhat detailed account of Dr. SHARKEY's case because it corresponds so closely with my own ; in both we have the kidneys apparently normal macroscopically, and in both we have considerable masses of growth in other organs. Both cases, again, are young subjects for malignant epithelial tumour.

Putting aside the question of the growth starting independently in the various sites of disease, two questions seem to present themselves—(1) Is the condition in the kidney congenital and not connected with the growths at all ? (2) If carcinoma of the kidney, is the condition primary or secondary ?

The widespread, in fact universal, deviation from the normal in the structure of the glomeruli seems to favour the congenital theory.

So far as I am aware, however, in the lower animals, and in the primitive renal tubule in Man, the epithelial lining consists of a single layer of cells. The increased cellular mass indicates, therefore, a new formation, and this is confirmed by the occasional penetration of the basement membrane by the proliferating epithelium.

The character of the cells of the new growth is of interest in connection with COHNHEIM's embryonic remnant theory of tumour formation. Assuming, then, that it is a question of growth, the nature of this, whether primary or secondary, arises. The extensive growth in the liver, lungs, etc., as compared with that in the kidney, does not of necessity prove the secondary character of the latter ; it seems to me, further, that if the kidney growth were secondary, that the epithelial growth would have been found over the vascular tuft as well as on the peripheral part of the capsule.

My conclusion is, therefore, that my case is one of primary carcinoma of the kidney arising in the glomeruli, and possibly associated with a persistence of the epithelium of the primitive renal tubule.

---

* Trans. 'Path. Soc.,' London, 1882, vol. xxxiii, p. 195.

TWO CASES OF LYMPHO-SARCOMA*

WITH REMARKS UPON THE DIFFERENTIAL DIAGNOSIS OF SOME GENERAL GLANDULAR ENLARGEMENTS

By JOHN HILL ABRAM, M.D. Lond., M.R.C.P., Assistant Physician Royal Infirmary, Assistant Lecturer on Pathology, University College Liverpool

The cases which I report were in the Royal Infirmary under my care in the latter part of 1897 and the earlier part of 1898.

Case I

J. M., æt. 41, sailmaker, was admitted on September 8th, 1897, for pains in the joints and glandular swellings. Apart from consumption in a collateral branch the family history was good.

He stated that his general health had always been good, and that he had never had any venereal disease. There was no scar on the penis. The swellings were first noticed in March, 1897, in the neck as usual; then the groins, and then the armpits. In June pains in the limbs were present. Whilst under treatment a rash appeared on the body, and afterwards on the face and head, attended with marked pruritus.

On Admission.—A well-built, powerful man. There is present thickly on the face, and more sparsely on the trunk and extensor aspects of the limbs, a raised, dusky-red rash, slightly scaly, but with no sign of vesication. It does not now itch.

Glands.—Marked swelling on both sides of the neck and face from the clavicle to the zygoma, also in the submaxillary triangles, the groins, and the axillæ. The glands can be separated from one another, and can be readily moved beneath the skin and on the deep tissues. Numerous isolated subcutaneous nodules are present over the trunk. The fingers and toes are swollen, and the skin thereof livid; they are very tender.

The tonsils are not enlarged. Dr. Hunt reports 'that a distinct swelling exists below the vocal cords, narrowing the lumen of the air-passage to a comparatively narrow slit.' The spleen reaches the costal margin. The liver is not enlarged.

R. 26. P. 84.

He has some dyspnœa, but can lie down. Some cough, with tough, blood-stained sputum. There is some dulness, with increased resistance behind the sternum. The respiratory movement is entirely abdominal. Chest emphysematous. Bronchitic sounds all over. Heart normal. Nervous system normal. Urine, 1024 acid, no abnormal constituents. Blood, Hh. 45 per cent. R.C. 3,600,000. Wh.C. 24,800. W : R : : 1 : 145 roughly.

---

* Reprinted from the 'Liverpool Medico-Chirurgical Journal,' July, 1899.

Leucocytes are mainly small lymphocytes. Eosinophil cells not excessive.

He was put on liq. arsenicalis *m* iii thrice daily after food, and this was gradually increased until, on October 9th, he was taking 12 minims thrice daily.

During this time he seemed to improve, the glands becoming smaller and softer, and the rash fading.

On October 15th some œdema of limbs and scrotum was observed, also slight albuminuria. The temperature rose to 103°. No rigour. The arsenic was omitted. It is noted on the 17th that he passed 172 oz. of urine without albumen.

A syringeful of blood was taken from a superficial arm vein, and a free growth of staphylococci was obtained. Blood now R.C. 3.5 million, Wh.C. 14,000, mainly polynuclear. On the 22nd the T. was again normal.

During the attack of fever the diminution in the size of glands was most marked, and far surpassed the slight change resulting from the exhibition of arsenic. He had a second febrile attack on November 8th lasting until November 15th, when blood showed R.C. 4,500,000, Wh.C. 25,000, still polynuclear in the main.

On November 18th glands again enlarging.

Without giving full details, I may say that the glands slowly increased, the rash became again prominent, and the dyspnœa severe. On December 8th the R.C. were 3,500,000, the Wh.C. 50,000, still mainly polynuclear.

He unfortunately contracted measles and died.

P.-M.—The lymphatic glands in the situations noted during life are greatly enlarged. In the mediastinum the glands are fused together, as is the case also in the lumbar glands. The spleen is enlarged, no nodules present. Nodules of growth are present in the liver and kidney on microscopical examination.

The bone marrow is normal.

The mucous membrane of the larynx and trachea shows a uniform thickening; in the arytæno-epiglottidean folds are two localized nodules the size of peas.

Histological examination of the lymphatic glands reveals an increased stroma, an accumulation of lymphoid cells, occasional giant cells, but no signs of necrosis. Eosinophil cells are present in some number.

Case II

Henry K., aged 28, porter, was admitted under my care on February 18th, 1898, complaining of cough. There is no special point in the family history. Has always been healthy. No history of venereal disease. His fatal illness began with a dry cough about Christmas, 1897, and the enlargement of the glands was first noticed in January, 1898. He is thin, ears cyanotic, cheeks flushed, skin over chest œdematous, temp. 100°.

The glands on both sides of the neck, the suboccipital glands, the axillary and inguinal groups are enlarged; in part the glands are fused, and somewhat fixed on the deep tissues. In one area a gland has become adherent to the skin, which is reddened.

Red corpuscles, 5,000,000; white, 31,000, mainly polynuclear. Eosinophil cells are not in excess of the normal. He has a troublesome cough, profuse frothy muco-purulent expectoration, no tubercle bacilli. There is some evidence of pressure upon the left bronchus.

Some dulness beneath manubrium sterni.

The remaining organs were normal.

The dyspnœa became severe, but Dr. HUNT reported the larynx and trachea to be normal.

This man, too, was treated with arsenic, but without avail, and he died suddenly on April 5th.

P.-M.—The morbid appearances are practically the same as those of Case I.

General enlargements of the lymphatic glands are not in my experience very common, and it has seemed to me that of late years they have become still less common.

The explanation of this increasing rarity, if it be true, is difficult, and I shall not attempt it, but it affords me one ground for bringing the subject forward. A second and more important feature is the obscurity which veils the exact pathology and diagnosis of these affections. In CLIFFORD ALLBUTT'S 'System of Medicine,' under the heading of Pseudoleukæmia, or HODGKIN'S Disease, I find the following synonyms given: anæmia lymphatica, anæmia splenica, lymphadenoma, lympho-sarcoma, cachexia without leukæmia, malignant lymphoma, desmoid carcinoma.

No doubt a certain number of cases have been of late severed from the category of pseudoleukæmia by the recognition of their specific infective cause, and this may, to some extent, account for the real or apparent diminution in the number of cases that I have already referred to.

The general enlargements of the glands which may still be confounded are the following: lymphadenoma, lympho-sarcoma, acute tuberculosis of the lymphatic glands, and lymphatic leukæmia.

I exclude from consideration true sarcoma of the lymphatic glands, and thus clearing the ground we are met with the question—Are lympho-sarcoma and lymphadenoma distinct diseases?

HAMILTON* states that they are essentially different, though often confounded. DRESCHFELD† holds that they are one, and he draws a very interesting and striking comparison between malignant lymphoma and the acute and chronic manifestations of tuberculosis.

The histological examination of glands from both cases—both from the clinical standpoint, I mean—affords us no help. In both cases we find an increase of the lymphoid cells, an overgrowth of fibrous tissue, and more or less numerous giant cells. The softer glands differ mainly from the harder variety in the greater number of leucocytes present. In both forms there is little or no tendency to caseation or necrosis, an important point in the differential diagnosis from tubercle of the glands.

A further point, if it be confirmed, is the statement by GOLDMANN,‡ that in malignant lymphoma eosinophil cells are present, whereas in tubercular glands they do not occur. These

---

* 'Text-book of Pathology,' vol. i.

† 'Brit. Med. Journal,' 1892, vol. i, 893; 'Deutsche Med. Wochensch.,' 1890.

‡ 'Centralbl. f. Allg. Path.,' 1892.

cells were present in both my cases (specimen exhibited). I return to the question of the identity of lympho-sarcoma and lymphadenoma.

From the clinical standpoint two features stand out which deserve careful investigation. In the first place the rapid enlargement of the glands in lympho-sarcoma leads to considerable periglandular inflammation, or, as others say, the growth infiltrates the surrounding tissue, and as a result the glands become fixed to one another, the deep tissues, and the skin.

In lymphadenoma, where the growth is slow, this does not occur, and the glands remain isolated and movable for months and years.

Again, in lymphadenoma there is no leucocytosis, except, of course, during the occasional pyrexial attacks, when it is polynuclear in type. In lympho-sarcoma a slight excess of leucocytes is constant, the greater number of them being mononucleated and small. It must be remembered that in lympho-sarcoma occasional pyrexial attacks are not uncommon, and in such attacks the mononuclear leucocytosis is replaced by a polynuclear variety. This is of interest in connection with the often noted diminution in the tumours during the rise of temperature. Both features were well seen in my first case.

A further point, also illustrated by my first case, is the occurrence of skin lesions, which, so far as I know, have not been noted in cases which we are accustomed to term lymphadenoma.

It will be seen that I agree rather with HAMILTON than with DRESCHFELD in distinguishing between lymphadenoma and lympho-sarcoma. The differential points I have enumerated may appear individually very trivial, but when combined they seem to me important. I agree, however, with DRESCHFELD in recognizing the resemblance shown by these formations to inflammatory formations rather than true new growth, and the suggestion arising therefrom of specific infective agents.

Although the specific agents have not been determined, yet the search for them has borne fruit, in that some cases resembling lymphadenoma in their clinical course and anatomical structure have been proved to be due to the *Bacillus tuberculosis*; and in others the pyrexial attacks, in some cases, at any rate, have been assigned to their true cause—a secondary infection. In my first case the pyrexial attack was due to a staphylococcic infection, secondary in character; and I think that in all probability the cases reported by TRAVERSA,* LANNOIS, and EROUX,† were similarly secondary infections.

KELSCH and VAILLARD‡ found a short motile bacillus, both in life and post-mortem; experimental inoculations gave a negative result.

DRESCHFELD§ has recorded a case in which a short bacillus was found post-mortem in the kidney; no cultivations, however, were made.

As I have said, therefore, the organisms of these affections yet await their discoverer.

Acute tuberculosis of the lymphatic glands is very rare, and usually presents rapid caseation and necrosis, with abundant periadenitis and skin implication. No direct observations, so far as I

---

* 'Riforma Med. Napoli,' 1893, t. ix, p. 96.
† 'Lyon Medic.,' 1890, No. 34.
‡ 'Annales de l'Instit. Pasteur,' 1890, t. iv, p. 276.
§ *Loc. cit.*

know, have been made upon the blood, but I should expect a polynuclear leucocytosis of the ordinary inflammatory type.

Lymphatic leukæmia is also a rare disease, and the marked excess of lymphocytes renders its diagnosis easy. The glands remain isolated, and the skin is not involved.

I would suggest that in all cases of general glandular enlargements we should adopt, in addition to the routine clinical investigation, a careful blood examination, histological and bacteriological, and further, excise a gland, not so much for histological as for experimental inoculation. In this way, I think, we may make some progress towards a better knowledge of this still vexed question.

In conclusion, I shall tabulate briefly the points which, in my opinion, should guide us at present in grouping our cases.

Acute tuberculosis of glands.—Matting of the glands, softening thereof and adhesion to the skin; an early and usual course. Leucocytosis of the ordinary inflammatory type. Lesions in the skin practically unknown.

Lymphadenoma.—The glands remain isolated and non-adherent for months and years. Blood shows simple anæmia without leucocytosis, except when T. present; then it is of the usual inflammatory type. Skin lesions not known.

Lympho-sarcoma.—Glands soon run together and infiltrate tissues. Leucocytosis of mononucleated cells. Skin lesions common.

Lymphatic leukæmia.—Glands remain isolated, leucocytosis well marked, mononuclear cells.

In all cases enlargement of spleen, anæmia, hæmorrhages, irregular fever, and various other symptoms may be present.

THE MORBID ANATOMY AND PATHOLOGY

OF DR. BRADSHAW'S CASE OF MYELOPATHIC ALBUMOSURIA*

By THOS. R. BRADSHAW, B.A., M.D., M.R.C.P, Senior Assistant Physician to the Liverpool Royal Infirmary

and

W. B. WARRINGTON, M.D., M.R.C.P., Demonstrator of Pathology in University College Liverpool, Assistant Physician to the Hospital for Consumption and Diseases of the Chest

Plate XIII

On the 26th of April, 1898, one of us (Dr. Bradshaw) read before this society an account of a case in which the urine contained a very large quantity of a form of proteid, which was shown by its chemical reactions to be related to the class of bodies known as albumoses.† The main facts of the case which were then reported, and its subsequent history until the death of the patient, were briefly as follows.

A man 70 years of age, a respectable shopkeeper in Liverpool, noticed about the end of the year 1896 that he passed milky urine from time to time. In August, 1897, systematic examinations of the urine were instituted, and it was found that it always contained a large but variable amount of a proteid body which was coagulated by a temperature below 60° C., and yielded other reactions which served to identify it with the peculiar albuminous body which was first described by Bence Jones in 1847, and is now commonly known as albumose.‡

Beyond the fact of the presence of this remarkable body in the urine there was at first nothing to suggest that the patient was the subject of any serious disease. All the ordinary indications of renal disorder were wanting, and for some months there was nothing in his circumstances or condition which afforded any clue to the significance of the remarkable condition of the urine. An examination of the literature, however, showed that the kind of albumosuria described by Bence Jones had been observed in six cases in all, and that in every instance it had been found to be associated with an affection of the bones of the trunk. This affection had been at first regarded as a form of osteomalacia; but the more recent observers had found that it was a form of multiple myeloma, a disorder in which the bones undergo absorption, and become attenuated in consequence of the development of a new growth which originates in the marrow

---

* Reprinted from the 'Medico-Chirurgical Transactions,' vol. lxxxii.

† T. R. Bradshaw. 'A Case of Albumosuria in which the Albumose was spontaneously precipitated.' 'Med.-Chir. Trans., 1898, p. 259.

‡ Bence Jones. 'On a new Substance occurring in the Urine of a Patient with Mollities Ossium.' 'Phil. Trans. of the Royal Society,' 1848.

spaces and spreads outwards, causing absorption of the hard parts of the bone. In the few instances in which albumosuria had been found in connection with multiple myeloma, the existence of the latter had only been recognised on the post-mortem table ; it had never been suspected during the patient's life ; but the records of these cases led Dr. BRADSHAW to suspect that this might be a case of multiple myeloma, and to watch carefully for the advent of local indications of bone disease. This suspicion was strengthened when, near the close of 1897, the patient complained of severe pains and tenderness about the ribs, sternum, and back, and it became almost a certainty when, early in 1898, there appeared obvious signs of diminished rigidity of the bones, consisting of an unnatural yielding of the chest walls to the pressure of the hand or the stethoscope, curvature of the spine, and subsequently spontaneous fractures of the ribs. During the progress of the case there were remarkable remissions in the intensity of the symptoms, both local and general. In January, 1898, the patient was unable to leave his bed on account of the pains, and suffered from a severe attack of bronchitis, followed in February by pneumonia affecting one lung and accompanied by rusty sputum. For some days he seemed to be *in extremis*. After this he gradually improved ; the pains subsided almost entirely, the lungs cleared up, and in the spring he went to reside with friends in Cheshire, and for two or three months was able to get up and down the stairs and to take short walks out of doors. About the middle of July the pains returned with great intensity, and he was unable to leave his bed ; deafness was noticed on one side, uncontrollable vomiting came on and he died, apparently from exhaustion, on August 10th. The peculiar character of the urine persisted till the end, but a specimen passed a few days before the fatal issue contained a little albumin in addition to the large quantity of albumose.

Owing to the death having taken place in a private house some valuable time was lost before arrangements could be made for removing the remains to a mortuary and holding an autopsy. A limited examination only was permitted, but it was amply sufficient to confirm the diagnosis made during life.

The following are the chief points of the necropsy, which was made by us 59 hours after death. There were also present Dr. JAMES BARR, Mr. W. T. THOMAS, and several other practitioners who were interested in the case.

Externally there were no indications of decomposition. Rigor mortis was present. On removing the integument from the thorax a small quantity of subcutaneous fat was found.

The whole bony framework of the thorax, the sternum, ribs, and vertebræ, presented a striking departure from the normal. The bones were so brittle that they could be broken by a slight pressure with the finger, a condition more noticeable in some spots than in others, and there were several complete fractures which had doubtless occurred during life. On the right side the VIth rib was found broken across about 3 inches from its cartilage, and there was an incomplete fracture of the VIIth rib in a corresponding situation. The VIIIth rib presented a slight irregularity which seemed to be due to a fracture which had united. On the left side the Vth and VIth ribs were broken about half an inch from their cartilages.

The change was very pronounced in the sternum, which broke while it was being removed. The gladiolus contained a large irregular cavity filled with a red mass resembling splenic pulp,

which was easily squeezed out by slight pressure on the bone. This cavity corresponded to the position of a spot of great tenderness which had been especially noticed several months before death.

There was a remarkable backward curve of the dorsal spine, and projection forward of the lumbar vertebræ and the promontory of the sacrum, constituting an extreme exaggeration of the normal curves. The degree of the curvature in the dorsal region was roughly measured by drawing a chord from the front of the body of the VIIth cervical vertebra to that of the IVth lumbar. The body of the IXth dorsal vertebra, where the backward projection was greatest, was 2 inches behind this line.

The body of the VIIth dorsal vertebra was removed; it was soft enough to be easily cut with the knife. The cut surface felt soft, and presented a disintegrated appearance not unlike that of splenic pulp.

The organs presented no striking abnormality. Those in the thorax were in their usual position; the pleuræ were free except for some slight adhesions at the apex; the right contained about 1 ounce of blood-stained serum, the left about 2 fluid drachms. There was no fluid in the pericardium. The lungs were normal. The heart weighed 8½ ounces; the right auricle contained a partly decolourized plot; the tricuspid orifice admitted three fingers; the left auricle was normal; the mitral orifice admitted two fingers; the valve appeared to be competent, but the curtains were thickened; the aortic valve was competent, and to all appearance healthy; there was slight atheroma at the beginning of the aorta.

Post-mortem changes were much advanced in the abdominal organs, especially the pancreas. The liver weighed 37 ounces, its surface was normal; there was no fatty change in its substance and no secondary growths; the hilum was free; the gall-bladder was filled with bile, and there were no gall-stones. The stomach, intestines, and spleen were to all appearance normal.

The right kidney weighed 2½ ounces. The capsule was rather too adherent, and on removal showed a whitish-yellow cortex mottled by distended capillaries. At the upper end a cyst the size of a hazel-nut was found, containing a glairy greenish fluid. At the lower end there was a similar cyst about the size of a pea. The cortex was much diminished, the pyramids were very white, the blood-vessels were not very obvious. On transverse section the outer third of the organ was seen to be occupied by a denser material of a yellowish-white colour. The left kidney weighed 3½ ounces. On section it resembled the right, but the departures from the normal were less apparent. On the convexity there was a cyst the size of a hazel-nut. The appearance of the organs led us to suppose that they were able to perform their functions fairly well until the death of the subject.

No enlarged glands were found anywhere, nor were any tumours observed either attached to bones or in any part of the body.

A piece of the upper end of one tibia was sawn of. It appeared to be quite normal.

An examination of the head was not allowed.

The material reserved for further examination consisted of a piece of a rib, of a dorsal

vertebra, and of the sternum, some of the gelatinous marrow-like substance in the sternum, and pieces of kidney.

On the same day some of the fresh marrow-line substance was extracted with a weak sodium chloride solution. The extract gave a marked albumose reaction with nitric acid and a turbidity with hydrochloric. Unfortunately the rest of the marrow-like material was by inadvertence placed in weak formalin solution, with the result that the proteids were rendered insoluble, and so their coagulation points could not be taken. As far as it went, the evidence pointed to the soft material in the bones containing the same substance as was found in the urine.

The piece of rib exhibited, preserved in formalin, shows that the bone is reduced to a mere shell about the thickness of notepaper, but its general outline is preserved. The interior is occupied by soft material, which, in the fresh state, was a bluish-red semi-solid pulp, which had taken the place of all except the most superficial parts of the bone (Pl. V, Fig. 1).

A small piece of rib, freed as far as possible from periosteum and other soft material, and dried in an incubator, was sent to Mr. COLLINGWOOD WILLIAMS, the Liverpool City Analyst, who kindly undertook the analysis of it for us.

His report is as follows:—

The quantity available for analysis was 0·117 gramme.

Organic matter	45·85	per cent.
Mineral	54·15	„
	100·00	„

DESCRIPTION OF PLATE XIII

FIG. 1. A portion of an affected rib near its cartilage. To the right is seen the cartilage in transverse section; to the left is seen the thin shell of bone, part of which has been removed so as to show the contained mass of new growth, which has shrunken somewhat and appears dark. *(Nearly natural size.)*

FIG. 2. Transverse section of decalcified rib. The osseous tissue is greatly reduced in thickness, and is being invaded by the new growth which is seen *in situ*. *(Zeiss. obj. A without ocular.)*

FIG. 3. Section of new growth in interior of sternum. The general characters of the structure are those of a round-celled sarcoma. *($\frac{1}{8}$ inch objective.)*

FIG. 4. The same as Fig. 3, more highly magnified, showing the cells of the growth in more detail. *(Apochromatic 3 mm. immersion objective, aperture 1.40, compensating ocular 12.)*

FIG. 5. The same as Fig. 4, another part of section.

FIG. 6. Transverse section of decalcified bony shell of rib.

(*i*) Inner aspect of shell, in contact with new growth which has become detached during manipulation.

(*e*) External surface, with muscle attached. This section gave some evidence of a retrograde change in the structure of the bone. ($\frac{1}{8}$ *inch objective.*)

FIG. 7. Transverse section of superficial layer of decalcified sternum.

(*b*) Internal surface of osseous layer, apparently undergoing erosion.

(*n*) Fragment of new growth *in situ*. (*Zeiss obj. A without ocular.*)

FIG. 8. Tangential section of dried bony shell of rib, prepared by grinding. Shows normal disposition of lacunæ and canaliculi. (*Zeiss obj. A without ocular.*)

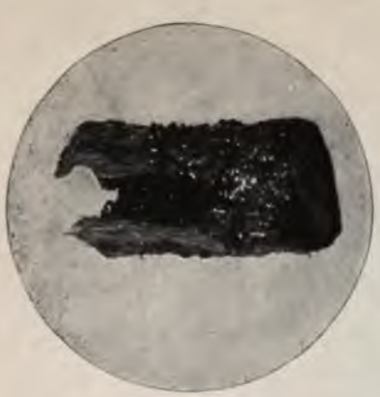


FIG. 1.

FIG. 2.

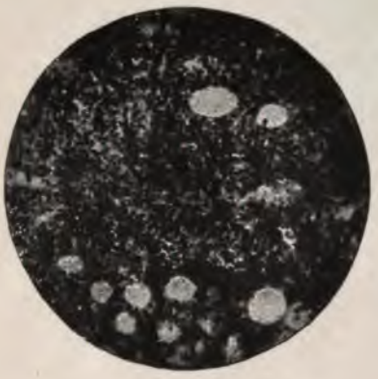


FIG. 3.

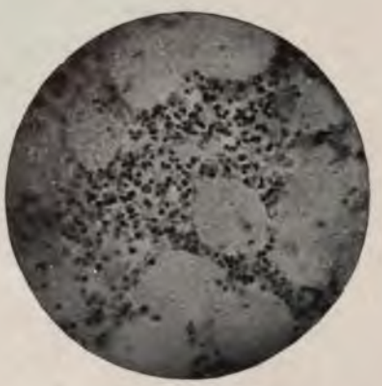


FIG. 4.

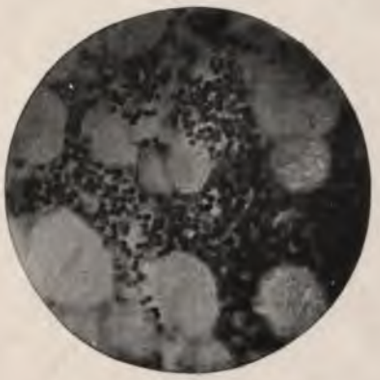


FIG. 5.

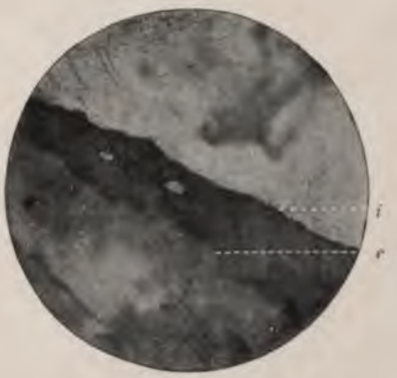


FIG. 6.

FIG. 7.

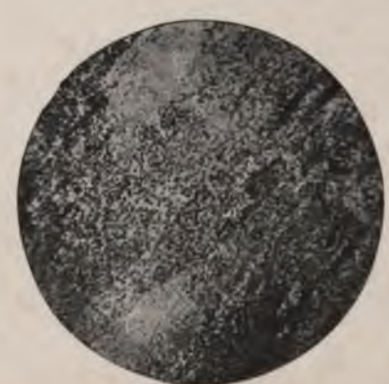


FIG. 8.

Adlard & Son, Imp.

Composition of ash :—

Ca ...	36·63	
PO_4 ...	55·59	
Insoluble in HCl ...	5·10	Proportion of CaO, to P_2O_5
Not estimated ...	2·68	5 : 4
	100·00	

If we make due allowance for the fact that no special precautions were taken to remove the fat, the proportion of organic to inorganic constituents does not depart greatly from the normal.

Microscopical Examination

The length of time which had elapsed since the death of the patient made the histological examination in some respects less satisfactory than we should have wished.

A piece of rib was preserved in 4 per cent. formol solution, and portions of the gladiolus and vertebra in Müller's fluid.

Thin slices of the dried bones were kindly prepared for us by Mr. J. A. Woods, L.D.S., and were mounted in Canada balsam.

The gelatinous marrow-like substance.—This was studied by means of cover-slip preparations fixed with absolute alcohol and ether, and stained in various ways, viz., methylene blue, eosin, and logwood and eosin, and also in sections; part of the material was fixed by saturated solution of perchloride of mercury, part in Müller's fluid, hardened in alcohol and embedded and cut in paraffin. The sections were then stained by the above-named dyes, and also by picro-carmine and borax-carmine.

The marrow-like substance was also studied *in situ* in sections of the bone which had been decalcified by means of a solution of phloroglucin in 5 per cent. nitric acid.

By these means it was found that the cancellous spaces of the ribs, sternum, and vertebra were densely packed by a round-celled growth. This growth invaded the bones along the vascular channels, which were considerably dilated, so that islets of bone appeared embedded in the mass of cells.

The cells were almost entirely of the lymphoid variety, a single large nucleus being alone visible in the majority.

A few oval or spindle cells were seen infrequently, but no myeloplaques or eosinophile cells were found. Red blood corpuscles appeared in considerable numbers, but were never observed to be nucleated. In addition to the lymphocytal form of cell, larger cells with a considerable amount of protoplasm and either a single, double, or reniform nucleus were met with (Pl. XIII, Figs. 2, 7, and 8).

The cover-glass preparations showed the same general features, including the absence of myeloplaques and eosinophile cells, but owing to the presence of fatty and albuminous material they were less satisfactory than the sections.

The Bone.—The decalcified bone of the sternum and vertebra showed normal features;

there was no transformation of bone into a lower type of connective tissue, such as has been observed in undoubted cases of osteomalacia, and there was an entire absence of osteoclasts. Dry preparations of these bones likewise showed a normal structure. On the other hand, in the rib, the delicate bony shell, which was cut in transverse section, appeared to have undergone retrograde changes; the normal lacunæ and canals were feebly represented, and where present were irregular in shape and distribution. On the inner surface they seemed to have entirely disappeared, and that part of the bone had a fibrous and at the same time dotted structure strongly suggestive of a reversion to a fibrous connective tissue. Indeed, the recognition of the tissue as bone would not have been easy had the facts of the case not been already known (Pl. XIII, Fig. 5).

Owing to the thinness of the bony shell it was impossible to prepare a dry transverse section of the rib so as to compare the inner and outer parts. A dry tangential section showed a perfectly normal arrangement of lacunæ and canaliculi (Pl. XIII, Fig. 6).

Kidneys.—The microscopic appearance was practically that met with in the milder forms of chronic interstitial nephritis; the connective tissues showed at places proliferation, and the cells of the tubules were found to be in a granular degenerated state, and occasionally lying free in the lumen.

Summary of the morbid anatomy.—As far as the limited examination which was permitted enabled us to ascertain, the characteristic lesions were confined to the bones of the trunk, but it is possible that the cranial bones were affected in a similar way. These lesions consisted in a disappearance of the osseous tissue, beginning centrally, and the growth in its place of a soft vascular mass of undifferentiated round cells. The remaining osseous tissue of the rib appears to have undergone retrograde changes, but that of the other bones shows normal characteristics, from which we infer that the primary lesion was the invasion by the cellular growth. There is no evidence to suggest the existence at any time of primary disease of the kidneys, the changes found in these organs being such as can be accounted for by the irritation arising from the passage through them of the abnormal material which they excreted for many months.

It is obvious that the histological characters of the growth in the interior of the bones merely show that it consists of embryonic cells which have undergone little or no differentiation, and are separated by a very small amount of intercellular substance. It might, therefore, be regarded, anatomically, either as a round-celled sarcoma, a lymphoma, or a granuloma. Clinically, its progressive development and fatal issue seem to entitle it to rank with the malignant neoplasms; but it differs from them in remaining strictly limited to one kind of structure and not spreading, either to contiguous parts or to distant organs. The cellular mass has originated apparently in the cancellous tissue or marrow spaces of the ribs, sternum, and vertebræ, and by its proliferation has caused absorption of the earthy salts and the proper tissue of the bone.

When we come to consider the pathological relationship of this condition we find that, apart from rickets, which only affects the growing bone, cases of extreme flexibility and brittleness of bone fall into two great divisions. On the one hand, we have those in which the essential pathological change is a simple absorption of the earthy salts—the Halisteresis Ossium of some

authors, a process which, when it affects the skeleton as a whole, constitutes the disease known as osteomalacia or mollities ossium. On the other hand, we have the cases in which absorption of the hard parts of the bone is due to its invasion by some kind of new growth, a condition which, when it occurs locally, is familiar to surgeons as myeloid sarcoma.

Within the last quarter of a century pathologists have come to recognise a more or less generalized absorption of the bones, arising from the simultaneous development of new growths in the bones in various parts of the body. This condition was first described as a distinct disease in 1873 by VON RUSTIZKY, of Kiew,* under the name of multiple myeloma, a designation under which several cases of multiple bone tumours have been recorded since. There can be no doubt that cases of multiple myeloma have been confounded with osteomalacia. The differential diagnosis between the two diseases is probably not always possible during life, but, as a rule, their clinical pictures are sufficiently distinct. Osteomalacia is almost confined to young women, often coming on in connection with pregnancy. All parts of the body are implicated—the extremities in a marked degree—with the result that the patient is soon confined to bed. The bones are often bent into most fantastic shapes, but actual fractures are few. Multiple myeloma, on the contrary, mostly occurs in men in the latter half of life; the bones of the thorax are those chiefly affected; the patient is able to leave his bed until near the end, deformities are not extreme, and fractures are common.

We have compared the microscopic sections in our case with sections from a case of undoubted mollities ossium which occurred in the Liverpool Royal Infirmary in 1880.† The sections were made by Mr. F. T. PAUL, who has kindly placed them at our disposal. In our case, as we have already shown, the interior of the bone is occupied by a cellular mass which is invading the hard parts. In Mr. PAUL'S specimens the cavities of the bone are occupied by what seems to be marrow, judging from the presence of large myeloplaques, and around this we find a layer of dense fibrous tissue, which seems to represent the bony tissue from which the salts have been removed. Briefly stated, it appears that in osteomalacia the bones are softened owing to loss of earthy salts; in multiple myeloma the osseous tissue is atrophied without any obvious change in its chemical composition. In the one case the bones bend, in the other they break.

The morbid anatomy of multiple tumours in the bones differs in different cases, and the records leave us uncertain whether the descriptions of multiple myeloma may not refer to two or more varieties of disease. There is, however, one circumstance which serves to differentiate these cases of bone disease into two distinct categories, namely, that some were attended by albumosuria and some were not.

In this communication we will confine ourselves to cases in which albumosuria was observed, and of these only six well-attested instances were on record when the present case was first communicated to this society in April last year. The leading features of these cases will now be considered.

---

* J. von Rustizky: 'Multiples Myelom. Deutsch. Zeitchr. f. Chirurgie', Band iii, s. 163, 1873.

† Specimens preserved in the Pathological Museum of University College Liverpool.

I. Macintyre and Bence Jones's case.* A description of the urine was communicated to the Royal Society by Bence Jones in 1847, and the whole case was brought before the Royal Medical and Chirurgical Society by Dr. Macintyre in 1850.

A man 45 years of age suffered from excruciating pains in the chest, back, and loins for upwards of a year. The progress of the disease was not uniform, but striking temporary improvement was noticed from time to time. There was tenderness on percussing the chest, but no deformity was observed. The urine contained the albuminous body which we now call albumose, and gave reactions which had never been recognized before, but which were described in detail in the paper read before this society last April, and need not now be repeated. There was no suspicion during life that the bones were the seat of any morbid affection.

At the post-mortem examination all the ribs were found to be soft and brittle. They could be readily cut with a knife, and could be broken by a very moderate amount of force. Their interior was charged with a gelatiniform substance of a blood-red colour and unctuous feel. The sternum and the bodies of the vertebræ were similarly affected, but the bones of the extremities were not affected, and the kidneys were healthy.

The microscopical examination of two lumbar vertebræ and a rib was made by Mr. Dalrymple, whose description corresponds very closely with our case.† He says, 'The disease appears to have commenced in the cancellated structure of the bone, for the external osseous laminæ are firmer and more healthy than the internal. The external are still hard, requiring the exertion of some force to cut them; they are thin, however, and when sliced expose large cancellous cavities filled with a red gelatiniform substance, threaded here and there by fine bony fibres.' The bulk of the gelatiniform tissue was composed of nucleated cells, but there were also present granular matter, oil globules, fat cells, caudate cells, and blood cells. It is worth noting that there is no mention of multinuclear myeloid cells. The condition was regarded as a form of mollities ossium; but Mr. Dalrymple makes the shrewd remark that it bears some resemblance to a malignant disease of the bone, and in the light of subsequent research we shall hardly be wrong in concluding that this was in reality a case of multiple myeloma.

II. The second case, Kühne's,‡ observed in 1867, was that of a man aged 40, whose urine contained the body described by Bence Jones. Deformities and other indications of an affection of the bones of the trunk were observed during life, and the diagnosis of osteomalacia was made, but no post-mortem examination was obtained.

III. The next case, which was investigated by Kahler and Huppert,§ was that of a medical man who died in 1887 at the age of 54, having been ill about 8 years. The symptoms were, for the most part, pain and tenderness, and, later, deformities in the bones of the trunk. Albumose was recognized in the urine for 6 years before the fatal termination.

---

* William Macintyre. 'Case of Mollities and Fragilitas Ossium accompanied with Urine strongly charged with Animal Matter. Med.-Chir. Trans.,' 1850, p. 211. Bence Jones, *loc. cit.*

† John Dalrymple. 'On the Microscopal Character of Mollities Ossium. Dublin Quarterly Journ. of Med. Sci.,' 1846, p. 85.

‡ W. Kühne. 'Ueber Albumose im Harne. Zeitschr. f. Biolog.,' N. F., Bd. i, 1883, s. 210.

§ O. Kahler. 'Zur Symptomatologie des Multiplen Myeloms: Beobachtung von Albumosurie. Prager med. Wochenschrift,' 1889, N. 4, 5. H. Huppert. 'Ein Fall von Albumosurie,' *ibid.*

The post-mortem examination showed the existence of softening of the ribs, sternum, and vertebræ. Microscopically the condition was found to be :—extreme osteoporosis with formation of numerous HOWSHIP's lacunæ, determined by the multiple proliferation of a tissue consisting of rather large round cells (round-celled sarcoma, so-called myeloma).

In this case several bony tumours were found in connection with various affected bones.

KAHLER's paper contains an exact clinical picture of the case. The diagnosis made during life was osteomalacia; but the author suggests that the occurrence of albumosuria might, in future, serve to distinguish multiple myeloma from osteomalacia.

IV. The next case was under the care of STOKVIS, of Amsterdam, and was published in 1891.* There was albumosuria but no indication of bone affection during life. After death, however, the bones were found highly brittle, and the place of the marrow was taken by a red gelatinous mass.

There were also in the connective tissue, the muscles, the periosteum, and the serous membranes numerous dry firm tumours of yellowish-white colour. Unfortunately, no microscopical examination was made, and it is quite possible that the tumours scattered about the body had nothing to do with the growth in the bones. In this case it is stated that the humerus was affected. The kidneys contained some chalky deposits, but appeared to be normal in other respects.

V. The next case was observed in the clinic of STINTZING, of Jena, in 1895.† The patient was a man aged 61, who presented marked deformity, the back being in a position of extreme kyphosis, and the head sunk between the shoulders. Pains in the back and the chest were severe. The urine was investigated with great care by Professor MATTHES,‡ of Jena, who came to the conclusion that the albumose which was present was really a body distinct from digestive albumoses, and, indeed, from all other bodies known to chemistry. The diagnosis made during life was osteomalacia. The post-mortem showed that there was extreme softening of the bones confined to the trunk, the enlarged marrow spaces being occupied by a dark bluish-red soft tissue. Microscopic examination of a piece of affected rib gave the following result :—

The compact tissue was wanting in places. Where it was present it was very much thinned, and contained numerous wide, irregular lacunæ, filled with a vascular spindle-celled tissue. Beneath the compact tissue, and replacing it where it was wanting, was a zone of tissue consisting of closely packed round cells, intersected by irregularly running bands of spindle-cells. It was of unequal thickness, and projected unevenly into the marrow. In it were some rather large islets of cartilage and numerous necrotic patches, which stained feebly and uniformly and were free from cells and lacunæ. The microscopic diagnosis was therefore given as chondro-sarcoma.

VI. All the foregoing cases were men. The sixth case was a woman aged 36, who was

---

* Zeehuisen. 'Ueber Hemialbumosurie. Jahresber. f. Thier-Chem.,' Bd. xxi, s. 412; Bd. xxii, s. 525; and Bd. xxiii, s. 577.

† Seegelken. 'Ueber Multiples Myelom und Stoffwechseluntersuchungen bei derselben. Deutsch. Archiv f. klin. Med.,' Bd. lviii, 1897, s. 276.

‡ M. Matthes. 'Ueber Eiweiss-Körper im Urine bei Osteomalacie. Verhandl. des Congress. f. innere Med.,' xix. Neumeister. 'Lehrbuch der phys. Chem.,' Aufl. ii, 1897, s. 804.

under the care of Professor SENATOR, and died in October, 1897.* She suffered from severe pains in the chest and back, and paralysis of one of the hypoglossal nerves. No deformities appear to have been noticed, and the diagnosis was nephritis with albumosuria. The post-mortem showed the presence of a new formation in several of the ribs proceeding from the medulla and regarded as a myelogenous round-celled sarcoma. The kidneys were amyloid.

A case reported by NOEL PATON and BYROM BRAMWELL was, at one time, believed by Professor HUPPERT to belong to this class. In the former paper the author (Dr. BRADSHAW) expressed doubts as to the correctness of this view, and quite recently Professor HUPPERT has abandoned it.†

In all these cases the condition of the bones was either altogether overlooked during life, or was considered to be osteomalacia. In KÜHNE's the absence of an autopsy leaves us in some doubt as to the actual condition of the bones, but it was probably the same as in the others.

It thus appears that our case is the first published case of albumosuria in which the diagnosis of multiple bone tumour was made during life; and further, that it is the first case in which the diagnosis made during life has been confirmed by the post-mortem examination.

Quite recently one of us (T. R. B.) has been informed by Dr. REGINALD H. FITZ, of Boston, of another case not yet published, in which the diagnosis was made during life and confirmed by the autopsy. A brief reference to this case will be found in a paper read before the Association of American Physicians last May.‡

The records of the six cases we have given in detail show a general resemblance in their morbid anatomy, and this resemblance is shared by the case we have described ourselves. The appearances are by no means identical, and we find in the records of cases where no albumosuria was observed descriptions of microscopical appearances indistinguishable from those described in the records of the cases we have quoted.§ This is, perhaps, to be expected, since round-celled formations may undergo various degrees of evolution, as we observe taking place in various kinds of sarcoma. It seems to us that the mere microscopical characters of the growths do not by themselves form any sound basis of classification; but we maintain that, in spite of their observed differences in structure, the occurrence of albumosuria entitles these cases to be placed in a category by themselves.

That a morbid process taking place in a region remote from the kidneys should be associated with the presence in the urine, for a long period, of large quantities of a substance which it does not contain in any other circumstances, is a rare event in pathology. The presence, however, in such a condition, of a substance not only foreign to normal urine, but unknown to the pathological chemist in any other connection, is probably without a parallel. The most obvious analogy, though it is only a remote one, is the occurrence of sugar in the urine in association with disease of the pancreas; but, in that case, we have only to deal with a familiar chemical substance which is

---

* Rosin. 'Ueber einen eigenartigen Eiweiss-Körper im Harne, &c. Berliner klin. Woch.,' 1897, No. 48.

† Huppert. 'Ueber den Nöel-Paton's chen Eiweiss-Körper. Centralb. f. d. med. Wissensch.,' 1898, No. 28.

‡ R. H. Fitz. 'The significance of Albumosuria in Medical Practice, &c. Amer. Journ. of the Med. Sciences,' July, 1898, p. 30.

§ F. Parkes Weber. 'General Lymphadenomatosis of Bones, &c. Journ. of Path. and Bact.,' January, 1898.

known to take part in the normal metabolism of the body. The occurrence of the albumose cannot be looked upon as an accident; when it is found at all it is present for months, or even years, and persists until the close of the case. We venture, therefore, to maintain that cases of bone disease with albumosuria must be placed in a different category from those in which this symptom does not occur; that albumosuria with bone disease is a distinct pathological entity, which is probably as far removed from other forms of multiple myeloma as the latter are from osteomalacia. If this be granted, it is desirable to give a name to the condition which will serve to mark its identity; and we offer the term *Myelopathic Albumosuria* as one which expresses the leading features of the disorder without implying the acceptance of any debatable theories as to its nature.

We may be permitted to recall the fact that we owe the earliest recognition of this disease to a Fellow of the Royal Medical and Chirurgical Society, and that it was before this society that the completed record of the first case was laid. Half a century elapsed before its occurrence was observed again in any part of the British dominions, and it seemed appropriate that when the second case appeared it should also be communicated to this society. But though history repeats itself, it never repeats itself exactly; and while the case presented fifty years ago was the first observed, the case which has been presented now is the first in which a correct diagnosis has been made during life and confirmed by examination after death. We venture to express the hope that the association of the society with the clinical history and the pathology of this remarkable disorder will not end here, but that before the next half-century has passed away one of the Fellows may be in the position to point out the causes which give rise to myelopathic albumosuria, and, perhaps, to indicate some means of arresting its course.

(For report of the discussion on this paper, see 'Proceedings of the Royal Medical and Chirurgical Society,' Third Series, vol. xi, p. 81.)

REPORTS

REPORT OF THE SCHOOL OF PHYSIOLOGY

YEAR 1899

Since the opening of the Department at the occasion of Lord LISTER's visit much has needed to be done to place the Laboratory in a position for full employment of its resources. Various fittings, incomplete at that time, have been now arranged and fixed. Lectures and practical classes now enjoy an accommodation and facilities they never before possessed with us. It has been gratifying to notice the increased ardour of the student, no doubt traceable to this cause. In addition to the teaching work and the considerable labour involved in adequately installing the rooms, some work of an investigational character has been proceeded with. There are, at present, however, no funds available to meet the expenses of such work. This is a matter of regret. The worker who is competent to pursue original investigation does not seem to expect to have to devote his own purse, as well as his own toil and time, to his subject. His view appears to me a fair one, and in no other country than our own is such a demand made from him. If he is capable and willing the expenses of his work, to the extent of providing him with the material and apparatus for its execution, are liberally and gladly provided by his University or the State, these, in most cases, being the same thing. We have, in the PHYSIOLOGICAL DEPARTMENT, had a number of offers from workers desirous of pursuing investigational study; but on hearing that the expenses must be met by themselves, even to the extent of supplying their own material and re-agents, the conditions have seemed too unfavourable for acceptance.

THE DEPARTMENT OF HISTOLOGY

The DEPARTMENT OF HISTOLOGY was more especialy under the charge of Mr. E. E. LASLETT, formerly Holt Fellow in Physiology. Mr. LASLETT during his tenure of the Assistant Lectureship, besides superintending the class work, prosecuted research. He published in the 'Lancet,' of August, 1898, a 'Note on a Modification of the WEIGERT-PAL Process of Staining applicable to Paraffin Sections.' Also he investigated, in conjunction with Dr. W. B. WARRINGTON, the pathological anatomy of a spinal case of much neurological interest. The results obtained in the work have appeared in 'Brain,' the Journal of the Neurological Society, under title 'Note on the Ascending Tracts in the Human Spinal Cord,' Winter No., 1899.

THE DEPARTMENT OF CHEMICAL PHYSIOLOGY

The DEPARTMENT OF CHEMICAL PHYSIOLOGY has been chiefly concerned with the instruction of students. Original investigation in this branch is particularly expensive, especially as in England—in contradistinction to continental countries—absolute alcohol cannot be obtained free of Excise duty for laboratory purposes.

On two occasions the advice of the Department has been sought regarding the composition of certain foodstuffs; and, obviously, it could be of practical assistance in many questions of municipal and commercial interest, *e.g.*, the composition and physiological value of new foodstuffs, fruits, &c., or the pharmacological value of samples of variable drugs like ergot, digitalis, cannabis indica, &c., or of new drugs.

THE DEPARTMENT OF PHYSICAL PHYSIOLOGY

The DEPARTMENT OF PHYSICAL PHYSIOLOGY has also devoted most of its attention to the students; but Mr. J. S. MACDONALD, the Assistant Lecturer on the subject, has also been engaged in active original research, the results of which, it is hoped, he will publish in an early number of these reports.

Most work has, however, been spent on the preparation for the systematic lectures, which have, indeed, been more of the nature of lecture-demonstrations. The time spent has been very considerable, but now that the course with its experiments has been once arranged, it will require to be varied only in small details from year to year, and much more leisure for research will be possible. Besides the ordinary course of lectures, another specially adapted for teachers has been given. For the first time also ladies, of whom 3 attended the ordinary and 1 the teachers' course, have been admitted to lectures on Physiology during this session.

REPORT OF THE SCHOOL OF PATHOLOGY

The beneficial effect of the increased accommodation given by the new laboratories has been very fully demonstrated during the past year. The Corporation of the City of Liverpool, and other public bodies, make full use of the increased facilities, and the School of Tropical Medicine is part of the Pathological Laboratory. The wiseness of devoting the major part of the Laboratory to research has been fully demonstrated by the number of senior workers who availed themselves of the great facilities placed for the first time at their disposal. Many friends of the College have been stimulated to help the work of the Laboratory.

Dr. ALEXANDER founded the Fellowship which has been named after him. Mr. WILLIAM JOHNSTON has still more recently founded a Colonial Fellowship, available for graduates of our Colonial Universities who wish to come to Liverpool to do research work in bacteriology. The practitioners of the district are taking more and more advantage of the special facilities offered them for pathological diagnosis, and the number of specimens examined has greatly increased during the past year. The Royal Infirmary and the Royal Southern Hospital have made arrangements whereby their bacteriological and pathological diagnostic work is done in the new laboratories.

The work of the Pathological School has been subdivided. Each separate department has its own Head of Department, and, as far as possible, its own separate service.

I. REPORT OF THE MUNICIPAL BACTERIOLOGICAL DEPARTMENT

The importance of systematic bacteriological analyses of the food and water supply has been long recognized; but the Corporation of the City of Liverpool was the first body in this country to appoint, in addition to the chemical analyst, another analyst—the present Professor of Pathology—whose duty it was to make regular bacteriological investigations. Other large cities are now following the example of Liverpool, and numerous smaller corporate bodies in the neighbourhood of Liverpool already largely make use of the Bacteriological Department of the THOMPSON YATES Laboratories.

The nature of the work undertaken for the City of Liverpool is as follows :—

1. Bacterial analysis of all food stuffs sent by the Medical Officer of Health.
2. Rabies inoculations.
3. Bacterial investigation of anthrax, glanders, choleraic diarrhœa and suspected cholera, yellow fever and plague, and other infective processes where bacterial examination is considered necessary by the Medical Officer of Health.
4. Special bacteriological researches which from time to time may be considered necessary.
5. Special diagnostic examinations required by the Medical Officer of Health, the City Hospitals, or, under special circumstances, by medical practitioners. These examinations include typhoid fever, diphtheria, tubercle, &c.
6. Special investigations in connection with the Port Sanitary and Hospitals Committee.

7. Daily, fortnightly, and monthly bacteriological analysis of the Liverpool water supply. Special investigations.

The work undertaken by the department is, therefore, partly routine and partly research; the opportunities for the latter being especially great in an important centre like Liverpool.

The following are the directions followed out in the Bacteriological Analytical Department :—

The Following to be Kept Ready to Hand

Nutrient agar stock tubes.

Carbolized (1-1000) nutrient agar stock tubes.

Nutrient gelatine stock tubes.

Glucose nutrient gelatine stock tubes.

Litmus milk tubes.

Pyrogallic acid, 1 oz. in 300 c.c. water, about 1 grm. in 10 c.c.

Normal caustic potash solution.

Sterilized Petri dishes.

Sterilized 1 oz. bottles with india-rubber stopper, for collecting samples of water.

Sterilized 12 oz. bottles with india-rubber stoppers, for collecting samples of milk.

Sterilized plugged test-tubes.

Sterilized pipettes.

The special agar water bath to be kept at 45° C. In this, agar tubes melted by placing them in boiling water are to be immersed, so that they may cool down to 45° C. and remain at that temperature ready for use. Gelatine tubes can be liquefied in this bath before use.

The special bath for keeping milk tubes at a temperature between 70° and 80° C. to be one-third filled with boiling water every morning, and cold water added, if necessary, till it falls to 80° C.; a small flame is then to be kept burning beneath.

To separate Spore-bearing Micro-organisms the inoculated tubes should be placed in the bath. A test-tube containing about 20 c.c. cold water is placed in the centre, and when the lid is put on the bulb of the thermometer should be immersed in the water. By this means the thermometer will show the temperature of the liquid inside the test-tubes.

The tubes should be left for 15 minutes after the thermometer registers 70° C. The temperature will never rise above 80° C., which is the boiling point of the Benzene placed inside the double jacket.

For Anaerobic Cultivations

Wide mouth bottles with glass stoppers or Buchner tubes to be used, according to the number of tubes to be incubated.

For *each* 200 c.c air capacity of the bottle or Buchner tube pour in 10 c.c. of the pyrogallic acid solution by means of a funnel, and, immediately after, 1 c.c. normal caustic potash solution for each 10 c.c. of pyrogallic acid solution used.

The tubes to be incubated anaerobically should be placed in the wide mouth bottle or Buchner tube before the mixture is poured in, and the stopper put on immediately after.

In using a large bottle it is better to let it remain in hot water, so as to expand the air before the mixture is poured in and the stopper closed; if this is not done the expansion of the air when the bottle is placed in the incubator is likely to raise the stopper.

Water Analysis

For Liverpool Corporation water, or water known to be pure.

Gelatine.—Add 1 c.c. to a gelatine tube liquefied in the bath at 45° C., and allowed to cool slightly. Pour into a sterilized Petri dish.

For a sample of water of unknown purity.

Make dilutions 1 in 10 and 1 in 100 before inoculating the gelatine tubes.

Count the number of colonies after 48 hours' incubation, put the plates back in the incubator, and examine again on the following day to make the final estimate of the number of liquefying colonies.

Carbolized Agar plates to be made in the same way as the gelatine plates. Examine these after 24-36 hours' incubation at 42° C. for the Colon bacillus. Look for and count any greyish translucent colonies like drops of oil.

Milk.—Add 1 c.c. water to a litmus milk tube, heat at 70° C. to 80° C. for 15 minutes, and anaerobically for 18-24 hours. If the culture 'goes' like a culture of Bacillus enteritidis sporogenes inoculate a Guinea-pig with 2 c.c. of the whey. (1 c.c. of whey for every 200 grs. weight of Guinea-pig).

Milk Analysis

With a sterilized pipette place 10 c.c. of the milk sample in a sterilized test-tube, and proceed in the same manner as in testing the water for Bacillus enteritidis sporogenes.

Carbolized Agar.—Add 1 c.c. of a 1-1,000 dilution of the milk sample to a carbolized agar tube, and proceed in the same manner as in testing the water for Bacillus coli.

5 c.c. of the milk sample are to be injected into a Guinea-pig subcutaneously. The animal is to be examined after 4 weeks for enlarged glands; if any are found examine the gland juice for the Tubercle bacillus.

The remainder of the milk sample is to be kept in the refrigerator till the test for tubercle has been settled.

Preserved Food Analysis

Tinned Meats or Fruits or Milk.

Note whether the top is concave.

Incubate the tin unopened at 37° C. for 3 days.

After 3 days' incubation make a small hole and note whether gas escapes, and, if so, the nature of the smell.

Make a larger opening in the lid and note the look of contents. Inoculate a milk tube, carbolized agar and ordinary agar tube, and proceed in the same manner as for water analysis.

Sterilized Milk and Tinned Milk.

Proceed as for ordinary milk.

Potted Meats or Foods not hermetically sealed.

Proceed as for tinned foods, but do not incubate first.

Shell-Fish

With sterilized fine-pointed pipette withdraw some of the fluid contents of the shell and inoculate—

(1) Carbolized agar tube.

(2) Litmus milk tube, and proceed as for milk and water.

With a second pipette remove some of the stomach contents, and proceed as above.

Raw Meats, Cooked Food, &c.

In all cases examine systematically for B. coli and B. enteritidis sporogenes.

Special investigations may, however, be necessary under special circumstances.

The number of samples taken for bacteriological analysis has increased very largely since 1898. In that year 438 samples were analyzed; in 1899, 664; and in the present year the minimum number will be 1,000. These figures do not include the cases of typhoid, diphtheria, and tubercle, which are very numerous. Every sample brought to the Laboratory is carefully entered on a special form, and a report furnished as soon as possible. The following are examples of the forms used for food analyses and diagnostic reports.

CITY OF LIVERPOOL No.

BACTERIOLOGICAL REPORT

Sample or case of - - - - (Sample number)
Where taken - - - - - - -
Date and time of taking - - - - - - -
By whom taken - - - - - - -
Description of sample or case - - - - -
Date and time of receipt at Laboratory - - - -
Date and time of investigation - - - - -
External temperature of atmosphere - - - -
METHOD EMPLOYED—
(*a*) Cultivation - - - - - -
(*b*) Inoculation - - - - - -
No. OF BACTERIA PRESENT—
Cultivated—At 21° C. - - - - -
At 37° C. - - - - -
Species present - - - - - - -
REMARKS— - - - - - - -

Date *Signed*

UNIVERSITY COLLEGE

CITY OF LIVERPOOL

FEVER HOSPITALS

No.

BACTERIOLOGIST'S REPORT
Hospital
Case of
Initials of patient
Age
Sex
Number of days ill
Date and time of receipt
Report
Date and time of despatch

THOMPSON YATES LABORATORIES, UNIVERSITY COLLEGE

Signature

REPORT TO THE MEDICAL OFFICER OF HEALTH OF LIVERPOOL OF THE SAMPLES ANALYZED DURING THE YEAR 1898

SAMPLES TAKEN FOR BACTERIOLOGICAL ANALYSIS OR EXAMINATION

New Milk	-	-	113
Condensed Milk	-	-	32
Oysters	-	-	23
Mussels	-	-	25
Cockles	-	-	22
Periwinkles	-	-	9
Whelks	-	-	1
Shrimps (potted)	-	-	2
Salmon (tinned)	-	-	18
Lobster „	-	-	5
Sardines „	-	-	8
Bloater Paste	-	-	2
Kipper Paste	-	-	1
Salmon and Shrimp Paste		-	2
Meat Paste	-	-	1
Gravy Soup	-	-	1
Boiled Rabbit (tinned)		-	1
Roast Mutton	-	-	1
Lunch Tongue	-	-	1
Turkey and Tongue		-	2
Chicken and Tongue		-	1
Ham and Tongue		-	1
Ham and Chicken	-	-	1
Potted Tongue	-	-	1
„ Veal and Ham	-	-	3
„ Beef	-	-	1
„ Ham	-	-	2
„ Strasburg Meat	-	-	1
Ribs of Beef	-	-	1
Lard	-	-	1
Water	-	-	1
Preserved Pineapple		-	2
„ Peas	-	-	3
„ Apricots	-	-	3
„ Tomatoes		-	3
„ Plums	-	-	1
„ Damsons	-	-	1
Jam	-	-	2
Golden Syrup	-	-	1
Treacle	-	-	1
Walnuts	-	-	1
Ice-cream	-	-	2
Samples of Hay	-	-	7
TOTAL	-		311

The total number of samples examined bacteriologically was 438. They may be divided into five classes, viz. :—

CLASS I. FOODS.—(*a*) Raw Foods.—Milk, Ice-cream, Shell Fish, etc.
(*b*) Preserved Foods.—Tinned Milks, Tinned and Potted Meats, and Preserved Fruits.

CLASS II. Water.

CLASS III. Special Bacteriological Investigations.

CLASS IV. Examination for Rabies.

CLASS V. Examination for Diphtheria and Typhoid (see page 214).

Class I—Food

Milk.—Samples of milk were sent to the bacteriologist mainly with a view to determine whether they were free from the tubercle bacillus.

The precautions taken in carrying on the investigation have already been detailed in previous reports, and it is not necessary to repeat them. It may be remarked that Professor Boyce, like the other bacteriologists who have carried on researches into the presence or absence of the tubercle bacillus, alludes incidentally to the important fact that in a certain proportion of cases the inoculated animal dies long before the time necessary for a tubercular lesion to develop. This result has been shown to be due to the presence in the milk of a virulent spore-bearing bacillus, possessing characters similar to those of the bacillus enteritidis sporogenes, and which is apparently associated with the choleraic disease so fatal to children during dry and hot weather.

The total number of samples of milk examined was 112, 28 being samples taken from the railway stations on arrival from the country, and 84 from the town. Of the 84 town samples 7 were proved to contain the tubercle bacillus, and of the 28 railway samples 5 were shown to contain the tubercle bacillus. These proportions of tubercular milk are in excess of the average.

With regard to ice-creams, the samples examined were found to contain a large number of micro-organisms, a circumstance which affords confirmatory evidence of the necessity for the measures obtained in the Liverpool Corporation Act of 1898, for ensuring cleanliness in the manufacture of ice-cream.

Milk

Sample Number.	Source.	Date 1898.	Tubercle bacilli present or absent.	Remarks.
150	Town	Jan. 4th	Absent	
154	Town	Jan. 14th	Absent	
155	Town	Jan. 14th	Absent	
156	Town	Jan. 14th	Absent	
160	Railway Station	Jan. 26th	Absent	
161	Railway ,,	Jan. 26th	Absent	
162	Railway ,,	Jan. 26th	Absent	
163	Grafton Street Hospital	Jan. 27th		Inoculated animal died Feb. 4th, from local abscess at seat of inoculation.
185	Town	Feb. 17th	Absent	
186	Town	Feb. 17th	Absent	
187	Town	Feb. 17th	Absent	
192	Railway Station	Feb. 23rd	Absent	
193	Town	Feb. 25th	Absent	
194	Town	Feb. 25th	Absent	
197	Town	Mar. 3rd		Animal died on March 10th from natural malnutrition.
198	Town	Mar. 3rd	Absent	
199	Town	Mar. 3rd	Present	
200	Town	Mar. 3rd	Absent	
208	Town	Mar. 8th	Absent	

MILK—*continued.*

Sample Number.	Source.	Date 1898.	Tubercle bacilli present or absent.	Remarks.
209	Town	Mar. 8th	Absent	
212	Town	Mar. 17th	Absent	
213	Town	Mar. 17th	Absent	
217	Railway Station	Mar. 24th	Absent	
218	Railway ,,	Mar. 24th	Absent	
219	Railway ,,	Mar. 24th	Absent	
220	Town	Mar. 25th	Absent	
225	Town	April 6th	Absent	
226	Town	April 6th		A large abscess developed at seat of inoculation, and animal died April 26th, 1898.
227	Town	April 6th	Absent	
228	Town	April 6th	Absent	
229	Park Hill Hospital	April 7th		Animal died within 24 hours. The milk was ropy, and contained a large number of organisms (non-pathogenic).
230	Park Hill Hospital	April 7th		Animal died.
237	Town	April 19th	Absent	
238	Town	April 19th	Absent	
241	Town	April 26th	Absent	
242	Town	April 26th	Present	
243	Town	April 26th	Absent	
244	Town	April 26th	Absent	
249	Town	May 5th	Absent	
250	Town	May 5th	Absent	
251	Town	May 5th	Absent	
252	Town	May 5th	Absent	
253	Town	May 6th	Absent	
256	Town	May 10th	Absent	
257	Town	May 10th	Absent	
258	Railway Station	May 12th	Absent	
259	Railway ,,	May 12th	Present	
281	Town	June 9th	Absent	
282	Town	June 9th	Absent	
283	Town	June 9th	Absent	
284	Town	June 9th	Absent	
295	Town	June 23rd	Absent	
296	Town	June 23rd	Absent	
297	Town	June 23rd	Absent	
298	Town	June 23rd		Animal found dead June 27th, 1898. Cause of death unknown.
312	Railway Station	July 7th	Absent	
313	Railway ,,	July 7th	Absent	
321	Railway ,,	July 21st	Absent	

MILK—*continued.*

Sample Number.	Source.	Date 1898.	Tubercle bacilli present or absent.	Remarks.
322	Railway Station	July 21st		Animal died on July 24th, 1898, from peritonitis and pleurisy.
327	Railway „	July 21st	Absent	
328	Railway „	July 26th	Absent	
330	Town	Aug. 6th	Absent	
331	Town	Aug. 6th	Absent	
336	Town	Aug. 12th	Absent	
337	Town	Aug. 12th	Absent	
342	Town	Aug. 18th	Absent	
343	Town	Aug. 18th		Inoculated animal died, but not from tubercle.
344	Town	Aug. 18th	Absent	
348	Railway Station	Aug. 26th	Absent	
356	Town	Sep. 7th	Absent	
359	Town	Sep. 7th	Absent	
363	Town	Sep. 16th	Absent	
364	Town	Sep. 16th	Absent	
365	Railway Station	Sep. 23rd	Absent	
366	Railway „	Sep 23rd	Absent	
371	Railway „	Sep. 30th	Absent	
376	Town	Oct. 6th	Absent	
377	Town	Oct. 6th	Absent	
380	Town	Oct. 12th		Inoculated animal died on Oct. 16th, 1898, with considerable subcutaneous extravasation.
381	Town	Oct. 12th	Absent	
382	Town	Oct. 12th	Absent	
385	Town	Oct. 21st	Absent	
386	Town	Oct. 21st		Animal died, but not from tubercle.
387	Town	Oct. 21st	Absent	
400	Railway	Nov. 7th		Animal died, but not from tubercle.
401	Railway Station	Nov. 7th		Animal died Nov. 11th, but not from tubercle.
402	Railway „	Nov. 7th	Absent	
409	Town	Nov. 17th	Present	
410	Town	Nov. 17th	Absent	
411	Town	Nov. 23rd	Absent	
412	Town	Nov. 23rd	Absent	
413	Town	Nov. 23rd	Absent	
414	Town	Nov. 23rd	Absent	
415	Town	Nov. 25th	Absent	
420	Railway Station	Nov. 30th	Present	
421	Railway „	Nov 30th	Present	
422	Railway „	Nov 30th	Present	

MILK—*continued.*

Sample Number.	Source.	Date 1898.	Tubercle bacilli present or absent.	Remarks.
423	Railway Station	Nov. 30th	Present	
434	Town	Dec. 16th	Absent	
435	Town	Dec. 16th	Present	
436	Town	Dec. 16th	Present	
437	Town	Dec. 16th	Present	
438	Town	Dec. 16th	Present	
441	Town	Dec. 22nd	Absent	
442	Town	Dec. 22nd		Animal died Dec. 31st, but not from tubercle.
443	Town	Dec. 29th	Absent	
444	Town	Dec. 29th		Animal died on Jan. 29th, but not from tubercle.
445	Town	Dec. 29th	Absent	
446	Town	Dec. 29th	Absent	
448	Railway Station	Dec. 30th	Absent	
449	Railway „	Dec. 30th	Present	

SHELL-FISH

Twenty-two samples of oysters, 22 of muscles, 23 of cockles, and 10 of periwinkles were examined bacteriologically. It is beyond dispute that these fish may be the means of transmitting certain forms of disease, notably typhoid fever. The most careful attention was given in investigating the micro-organisms which the samples were found to contain, special importance being attached to those groups of organisms which might be taken to indicate sewage contamination. Although the typhoid bacillus was not isolated in any of the samples, the bacillus coli, which is commonly associated with sewage contamination, was isolated in 15 per cent. of the samples; and in the cases in which special search was made for the bacillus enteriditis sporogenes this bacillus was found. The full details of the methods employed are set forth in the Reports already published by Professor BOYCE and Professor HERDMAN to the British Association, 1895-6-7-8. In the same Report will be found the reasons which led up to the following recommendations, which were made by those gentlemen, as to the means to prevent oyster beds, &c., from being contaminated with sewage :—

(A) That the necessary steps should be taken to induce the oyster trade to remove any possible suspicion of sewage contamination from the beds and layings from which oysters are supplied to the market. This could obviously be effected in one of two ways—either (1) by restrictive legislation and the licensing of beds only after due inspection by the officials of a government department; or (2) by the formation of an association amongst the oyster-growers and dealers themselves, which should provide for the due periodic examination of the grounds, stores, and stock by independent properly qualified inspectors. Scientific assistance and advice given by such independent inspectors would go far to improve the

condition of the oyster beds and layings, to re-assure the public, and to elevate the oyster industry to the important position which it should occupy.

(B) Oysters imported from abroad (Holland, France, or America) should be consigned to a member of the 'Oyster Association,' who should be compelled by the regulations to have his foreign oysters as carefully inspected and certificated as those from his home layings. A large proportion of the imported oysters are, however, deposited in our waters for such a period before going to market that the fact of their having originally come from abroad may be ignored. If this period of quarantine were imposed upon all foreign oysters a great part of the difficulty as to inspection and certification would be removed.

(C) The grounds from which mussels, cockles, and periwinkles are gathered should be periodically examined by scientific inspectors in the same manner as the oyster beds. The duty of providing for this inspection might well, we should suggest, be assumed by the various Sea Fisheries Committees round the coast.

The samples examined are indicated in the following table :—

Table I—Oysters

Sample number.	Date 1898.	Colon bacillus present or absent.	Remarks.
147	Jan. 4th	Absent	
174	Feb. 3rd	Absent	Actively motile bacillus isolated, no gas, no coagulation, thick growth on potato, no indol, alkaline reaction.
175	Feb. 8th	Absent	
178	Feb. 8th	Absent	
184	Feb. 12th	Absent	
204	Mar. 3rd	Absent	
207	Mar. 8th	Absent	
216	Mar. 18th	Absent	Actively motile bacillus isolated, gas forming, no indol, no coagulation, thick growth upon potato, alkaline reaction in neutral litmus whey.
232	April 14th	Absent	
235	April 14th	Absent	
248	April 28th	Absent	
255	May 6th	Absent	
269	May 20th	Absent	
278	June 3rd	Absent	
289	June 10th	Absent	
345	Aug. 22nd	Present	
391	Oct. 27th	Present	
397	Nov. 4th	Present	
427	Dec. 5th	Absent	B. enteritidis present.
428	Dec. 5th	Absent	
429	Dec. 5th	Absent	
439	Dec. 22nd	Absent	

Table II—Mussels

Sample number.	Date 1898.	Colon bacillus present or absent.	Remarks.
145	Jan. 4th	Absent	
146	Jan. 4th	Absent	
152	Jan. 11th	Absent	
157	Jan. 18th	Absent	Gas forming coccus isolated.
158	Jan. 18th	Absent	Gas forming coccus isolated.
171	Feb. 3rd	Absent	
172	Feb. 3rd	Absent	
177	Feb. 8th	Absent	Actively motile bacillus isolated, no gas, no coagulation, no indol, thick growth on potato, alkaline reaction.
180	Feb. 12th	Absent	
201	Mar. 3rd	Absent	Gas forming coccus isolated.
202	Mar. 3rd	Absent	Small actively motile bacillus isolated, no gas, no coagulation, no indol formation, thick growth on potato, neutral reaction.
205	Mar. 8th	Absent	
214	Mar. 18th	Absent	Gas forming coccus isolated.
215	Mar. 18th	Absent	
222	Mar. 30th	Absent	Actively motile bacillus isolated, gas forming, milk coagulated, no indol, abundant growth on potato, alkaline reaction.
240	April 21st	Absent	
246	April 28th	Absent	
350	Sept. 2nd	Present	
370	Sept. 23rd	Present	
384	Oct. 14th	Absent	
392	Oct. 27th	Absent	
398	Nov. 4th	Present	
417	Nov. 23rd	Present	
430	Dec. 5th	Absent	B. enteritidis present.
440	Dec. 22nd	Absent	

Table III—Cockles

Sample number.	Date 1898.	Colon bacillus present or absent.	Remarks.
148	Jan. 4th	Absent	
149	Jan. 4th	Absent	
153	Jan. 11th	Absent	
159	Jan. 18th	Absent	
173	Feb. 3rd	Absent	
176	Feb. 8th	Absent	
183	Feb. 12th	Absent	
196	Feb. 25th	Absent	
203	Mar. 3rd	Absent	
206	Mar. 8th	Absent	
221	Mar. 30th	Absent	Motionless bacillus isolated, no gas, no coagulation, no indol.

COCKLES—*continued.*

Sample number.	Date 1898.	Colon bacillus present or absent.	Remarks.
231	April 14th	Absent	
233	April 14th	Absent	Gas forming coccus isolated.
239	April 21st	Absent	
247	April 28th	Absent	
254	May 6th	Absent	
268	May 20th	Absent	
277	June 3rd	Absent	
285	June 10th	Absent	
349	Sep. 2nd	Present	
369	Sep. 23rd	Present	
399	Nov. 4th	Present	
418	Nov. 25th	Present	

Table IV—PERIWINKLES

Sample number.	Date 1898.	Colon bacillus present or absent.	Remarks.
179	Feb. 11th	Absent	
181	Feb. 12th	Absent	
182 (Whelks)	Feb. 12th	Absent	Bacillus, gas forming, no coagulation, no indol, neutral reaction.
195	Feb. 25th	Absent	
234	April 14th	Absent	
245	April 28th	Absent	
286	June 10th	Present	
288	June 10th	Absent	
383	Oct. 14th	Absent	
426	Dec 5th	Absent	B. enteritidis present.

B.—PRESERVED FOODS

Condensed Milk.—The temperature at which tinned foods is put up is usually supposed to lead to their complete sterilization. The bacteriologist's observations show that, on the contrary, many examples of tinned foods are far from sterile, and that, therefore, they may be the carriers of pathogenic organisms.

Thirty-four samples of various brands of condensed milks were examined bacteriologically, and out of these only one appeared free from micro-organisms.

In the others the number of organisms varied, being very abundant in some samples. No pathogenic forms were found. These observations are of importance, because no article of food of the nature of milk or meat should be tinned without making absolutely certain that it is sterilized. Spore-bearing bacilli similar to the bacillus enteriditis sporogenes can develop in the sealed tins if sterilization is not complete, and lead to the production of irritant and harmful products. If condensed milk is not sterile it has no advantage over raw milk from a bacteriological point of view.

NOTE.—The term pathogenic is used to denote those organisms which produce disease in man.

CONDENSED MILK

Sample number.	Brand.	Date 1898.	Remarks.
260	Head Brand	May 14th	Not Sterile.
261	Nestle's	May 14th	Not Sterile.
266	Calf Brand	May 20th	Not Sterile.
267	Table Brand	May 20th	Not Sterile.
270	Head Brand	May 27th	Not Sterile.
271	Nestle's	May 27th	Not Sterile.
272	Irish	May 27th	Not Sterile.
273	Magpie	May 27th	Not Sterile.
274	Nestle's	June 3rd	Not Sterile.
275	Cup	June 3rd	Not Sterile.
279	Hawthorne	June 3rd	Not Sterile.
280	Tip-top	June 3rd	Not Sterile.
293	Anglo-Swiss	June 16th	Not Sterile.
294	...	June 16th	Not Sterile.
299	Flag	June 25th	Not Sterile.
300	Cruiser	June 25th	Not Sterile.
306	Family	July 1st	Not Sterile.
307	Royal Standard	July 1st	Not Sterile.
308	Nestle's	July 1st	Not Sterile.
309	Calf	July 1st	Not Sterile.
310	Tip-top	July 1st	Not Sterile.
311	Head	July 1st	Not Sterile.
314	...	July 8th	Not Sterile.
329	Swan	July 26th	Not Sterile.
338	Daily	Aug. 13th	Not Sterile.
339	White Star	Aug. 13th	Not Sterile.
340	Nurse	Aug. 16th	Not Sterile.
341	Polo	Aug. 16th	Not Sterile.
346	Tantivy	Aug. 25th	Not Sterile.
347	Jersey Cow	Aug. 25th	Not Sterile.
372	...	Sep. 30th	Not Sterile.
404	Crow	Nov. 9th	Not Sterile.
416	Anglo-Irish	Nov. 25th	Not Sterile.
447	Ideal	Dec. 29th	Sterile.

Tinned Salmon.—Seventeen samples were examined, agar plate cultivations being made from each. The majority proved sterile, only three samples giving rise to colonies of bacteria upon the plates.

TINNED SALMON

Sample number.	Brand.	Date 1898.	Remarks.
164	...	Feb. 3rd	Sterile.
224	...	Mar. 30th	Sterile.
236	...	April 19th	Sterile.
276	...	June 3rd	Sterile.
287	...	June 10th	Sterile.
305	...	June 29th	Sterile.
315	Silver Crown	July 8th	Sterile
316	Gem	July 8th	Decomposed

TINNED SALMON—*continued.*

Sample number.	Brand.	Date 1898.	Remarks.
317	Royal York	July 16th	Sterile.
351	Puritan	Sep. 2nd	Sterile.
352	...	Sep. 2nd	Sterile.
354	Cycle	Sep. 7th	Sterile.
361	Signal	Sep. 14th	Sterile.
375	...	Oct. 1st	Not Sterile.
378	Cleeves	Oct. 7th	Sterile.
388	English Crown	Oct. 22nd	Not Sterile.
408	...	Nov. 17th	Sterile.

Tinned Sardines.—Nine samples were examined, and five were shown to be not sterile.

TINNED SARDINES

Sample number.	Brand.	Date 1898.	Remarks.
223	...	Mar. 30th	Not Sterile.
290	...	June 10th	Sterile.
318	International	July 16th	Sterile.
353	...	Sep. 2nd	Not Sterile.
360	...	Sep. 14th	Sterile.
373	...	Oct. 1st	Sterile.
374	...	Oct. 18th	Not Sterile.
389	...	Oct. 22nd	Not Sterile.
396	...	Oct 29th	Not Sterile.

Tinned Lobster.—One sample out of five examined was found to be not sterile.

TINNED LOBSTER

Sample number.	Brand.	Date 1898.	Remarks.
165	...	Feb. 3rd	Sterile.
191	...	Feb. 18th	Sterile.
304	...	June 29th	Sterile.
355	...	Sep. 7th	Not Sterile.
407	...	Nov. 17th	Sterile.

Miscellaneous Tinned and Potted Meats.—As the table shows, these were very varied and amounted to 29 samples; they included tinned meats, potted meats, meat pastes, and lard. Sixteen of the samples contained micro-organisms, and were therefore liable to undergo decomposition and to prove a source of danger.

Preserved Fruits.—What has been said as regards the importance of tinned meats holds equally good in the case of preserved fruits. It is essential that every care should be taken to see that they are put up completely sterile. The loss to the manufacturer from the preserved fruits and jams undergoing fermentation, and the unwholesomeness of such articles of food, could be prevented by more scientific methods of preparation. Out of the 15 samples examined, six contained micro-organisms of various kinds.

Fresh Fruits.—One sample of sprouting and fermenting fresh walnuts was examined. What has been said concerning the unwholesomeness of non-sterilized preserved fruits, is equally applicable to the unsound ripe fruit caused by the presence of organisms.

PRESERVED FOODS—MISCELLANEOUS

Sample number.	Brand.	Date 1898.	Remarks.
166	Lunch Tongue	Feb. 3rd	Sterile.
167	Preserved Pine Apple	Feb. 3rd	Sterile.
168	Preserved Apricots	Feb. 3rd	Sterile.
169	Preserved Tomatoes	Feb. 3rd	Sterile.
170	Preserved Pears	Feb. 3rd	Sterile.
188	Potted Tongue	Feb. 18th	Not Sterile.
189	Turkey and Tongue	Feb. 18th	Sterile.
190	Chicken and Tongue	Feb. 18th	Sterile.
210	Potted Veal and Ham	Mar. 12th	Not Sterile.
211	Bloater Paste	Mar. 12th	Not Sterile.
262	Kipper Paste	May 20th	Not Sterile.
263	Veal and Ham	May 20th	Sterile
264	Meat Paste	May 20th	Sterile.
265	Salmon and Shrimp	May 20th	Sterile.
291	Apricots	June 15th	Sterile.
292	Tomatoes	June 15th	Sterile.
301	Gravy Soup	June 29th	Sterile.
302	Pears	June 29th	Sterile.
303	Golden Syrup	June 29th	Sterile.
319	Boiled Rabbit	July 16th	Not Sterile.
320	Roast Mutton	July 16th	Not Sterile.
323	Veal and Ham	July 23rd	Not Sterile.
324	Ham and Chicken	July 23rd	Not Sterile.
325	Potted Ham	July 23rd	Not Sterile.
326	Turkey and Tongue	July 23rd	Not Sterile.
332	Potted Ham	Aug. 6th	Sterile.
333	Strasburg Meats	Aug. 6th	Sterile.
334	Potted Beef	Aug. 6th	Sterile.
335	Ham and Tongue	Aug. 6th	Not Sterile.
357	Bloater Paste	Sep. 7th	Not Sterile.
358	Salmon and Shrimp	Sep. 7th	Not Sterile.
362	Apricots	Sep. 14th	Sterile.
367	Ice Cream	Sep. 23rd	Not Sterile.
368	Ice Cream	Sep. 23rd	Not Sterile.
379	Ribs of Beef	Oct. 7th	Sterile.
390	Pine Apple	Oct. 22nd	Not Sterile.
393	Peas	Oct. 29th	Not Sterile.
394	Tomatoes	Oct. 29th	Not Sterile.
395	Treacle	Oct. 29th	Not Sterile.
405	Blackberry Jam	Nov. 9th	Not Sterile.
406	Rhubarb Jam	Nov. 9th	Sterile.
419	Lard	Nov. 25th	Not Sterile.
424	Plums	Dec. 1st	Not Sterile.
425	Damsons	Dec. 1st	Not Sterile.
431	Walnuts	Dec. 5th	Undergoing fermentation.
432	Potted Shrimps	Dec. 7th	Not Sterile.
433	Potted Shrimps	Dec. 8th	Not Sterile.

Class II

Water supplied for ordinary purposes—Systematic fortnightly and monthly examinations of the drinking water have been made. The samples of water are collected by a member of the bacteriologist's staff in small sterilized bottles, and plate cultivations made as quickly as possible after collecting. The analyses show that the water supplied to the city is very good. No pathogenic forms have been isolated, but numerous other non-pathogenic forms, such as would have been expected in wholesome water, have been met with. These are kept and propagated in the laboratory, and are always useful for reference.

The following are the sources which have been examined. :—

Fortnightly Examinations—

Ashton Hall Tap.

Fortnightly Examinations—

Wells— { Windsor Well. / Dudlow Lane Well. / Green Lane Well.

Prescot— { Vyrnwy Water. / Rivington Water. / Mixed Water.

Water—Fortnightly Samples.

Source.	Date 1898.	Time of collecting.	Time of examination.	Bacteria present. Gelatine. per c.c.	Agar. per c.c.
Ashton Hall Tap	Jan. 24th	5 30 pm.	5 30 pm.	46	4
Ashton Hall Tap	Feb. 7th	11 am.	11 am.	60	14
Ashton Hall Tap	Feb. 20th	2 pm.	2 pm.	14	1
Ashton Hall Tap	Mar. 7th	11 30 am.	11 30 am.	24	—
Ashton Hall Tap	Mar. 15th	5 pm.	5 pm.	60	2
Ashton Hall Tap	Mar. 6th	2 pm.	2 pm.	60	4
Ashton Hall Tap	April 18th	11 am.	11 am.	80	6
Ashton Hall Tap	April 25th	3 pm.	3 pm.	30	14
Ashton Hall Tap	May 15th	11 am.	11 am.	50	8
Ashton Hall Tap	May 27th	12 am.	12 am.	94	14
Ashton Hall Tap	June 10th	11 am.	11 am.	92	17
Ashton Hall Tap	June 22nd	3 pm.	3 pm.	27	11
Ashton Hall Tap	July 25th	10 am.	10 am.	100	—
Ashton Hall Tap	Aug. 6th	9 30 am.	9 30 am.	30	8
Ashton Hall Tap	Aug. 18th	3 pm.	3 pm.	33	—
Ashton Hall Tap	Sep. 3rd	10 am.	10 am.	54	17
Ashton Hall Tap	Sep. 17th	11 am.	11 am.	140	170
Ashton Hall Tap	Sep. 26th	12 am.	12 am.	36	14
Ashton Hall Tap	Oct. 15th	10 am.	10 am.	40	6
Ashton Hall Tap	Oct. 25th	9 am.	9 am.	70	4
Ashton Hall Tap	Nov. 12th	9 am.	9 10 am.	120	27
Ashton Hall Tap	Nov. 25th	2 pm.	2 10 pm.	6	—
Ashton Hall Tap	Dec. 19th	4 pm.	4 5 pm.	3	31

WATER—Monthly Samples.

Source.	Date 1898.	Time of collecting.	Time of examination.	Bacteria present. Gelatine. per c.c.	Agar. per c.c.
Dudlow Lane	Jan. 31st	1 45 pm.	5 pm.	120	4
Dudlow Lane	Mar. 4th	2 45 pm.	5 pm.	22	—
Dudlow Lane	April 29th	1 40 pm.	3 pm.	72	12
Dudlow Lane	July 12th	11 am.	12 am.	224	—
Dudlow Lane	Aug. 30th	10 30 am.	11 30 am.	500	7
Dudlow Lane	Sep. 27th	10 am.	1 pm.	44	100
Dudlow Lane	Oct. 25th	3 30 pm.	4 30 pm.	15	6
Dudlow Lane	Nov. 24th	2 30 pm.	3 30 pm.	35	56
Dudlow Lane	Dec. 19th	10 35 am.	12 am.	5	4
G. Holt Well (Green Lane)	April 29th	2 30 pm.	3 pm.	50	—
G. Holt Well „	Aug. 31st	10 am.	11 am.	100	20
G. Holt Well „	Sep. 27th	11 30 am.	1 pm.	16	12
G. Holt Well „	Oct. 26th	3 30 pm.	4 pm.	200	120
G. Holt Well „	Dec. 19th	11 am.	12 am.	18	1
Green Lane Well	Jan. 31st	2 35 pm.	5 pm.	*4000	*200
Green Lane Well	Mar 4th	3 pm.	5 pm.	30	—
Green Lane Well	May 26th	3 pm.	4 20 pm.	26	9
Green Lane Well	Aug. 6th	12 30 pm.	1 30 pm.	60	6
J. Holmes Well (Green Lane)	April 29th	2 30 pm.	3 pm.	896	—
J. Holmes Well „	Aug. 31st	10 am.	11 am.	40	15
J. Holmes Well „	Sep. 27th	11 30 am	1 pm.	18	10
J. Holmes Well „	Oct. 26th	3 30 pm.	4 pm.	100	300
J. Holmes Well „	Nov. 25th	10 40 am	11 35 am	122	230
J. Holmes Well „	Dec. 19th	11 am.	12 am.	20	10
Mixing Well (Prescot)	Mar. 4th	3 45 pm.	5 pm.	30	—
Mixing Well „	May 24th	11 30 am.	2 30 pm	30	10
Mixing Well „	July 7th	2 pm.	4 pm.	40	10
Mixing Well „	Aug. 5th	3 pm.	5 pm.	200	40
Mixing Well „	Aug. 29th	11 am.	1 pm.	300	100
Mixing Well „	Sep. 26th	2 pm.	3 30 pm.	200	180
Mixing Well „	Oct. 26th	11 30 am.	12 15 pm.	26	30
Mixing Well „	Nov. 23rd	11 30 am.	12 30 pm.	330	70
Mixing Well „	Dec. 19th	2 35 pm.	4 pm.	12	30
Rivington Aqueduct	Jan. 31st	3 30 pm.	5 pm.	6	—
Rivington Aqueduct	Mar. 4th	3 45 pm.	5 pm,	20	—
Rivington Aqueduct	Mar. 31st	1 pm.	3 pm.	44	6
Rivington Aqueduct	April 26th	2 pm.	5 pm.	60	—
Rivington Aqueduct	May 24th	11 30 am.	2 30 pm.	18	4
Rivington Aqueduct	July 7th	2 pm.	4 pm.	21	5
Rivington Aqueduct	Aug. 5th	3 pm.	5 pm.	30	2
Rivington Aqueduct	Aug. 29th	11 am.	1 pm.	12	6
Rivington Aqueduct	Sep. 26th	2 pm.	3 30 pm.	20	22
Rivington Aqueduct	Oct. 26th	11 30 am.	12 15 pm.	70	100
Rivington Aqueduct	Nov. 23rd	11 30 am.	12 30 pm.	450	33
Rivington Aqueduct	Dec. 19th	2 30 pm.	4 pm.	6	9
Vyrnwy Aqueduct	Jan. 31st	3 30 pm.	5 pm	6	—

* Harmless organisms: the increase due to incidental disturbances of the source at time of taking the sample.

WATER—Monthly Samples—*continued.*

Source.	Date 1898.	Time of collecting.	Time of examination.	Bacteria present. Gelatine. per c.c.	Agar. per c.c.
Vyrnwy Aqueduct	Mar. 4th	3 45 pm.	5 pm.	10	—
Vyrnwy Aqueduct	Mar. 31st	1 pm.	3 pm.	60	30
Vyrnwy Aqueduct	April 26th	2 pm.	5 pm.	6	—
Vyrnwy Aqueduct	May 24th	11 30 am.	2 30 pm.	6	6
Vyrnwy Aqueduct	July 7th	2 pm.	4 pm.	11	7
Vyrnwy Aqueduct	Aug. 5th	3 pm.	5 pm.	30	8
Vyrnwy Aqueduct	Aug. 29th	11 am.	1 pm.	30	12
Vyrnwy Aqueduct	Sep. 26th	2 pm.	3 30 pm.	42	34
Vyrnwy Aqueduct	Oct. 26th	11 30 am.	12 15 pm.	4	76
Vyrnwy Aqueduct	Nov. 23rd	11 30 am.	12 30 pm.	24	7
Vyrnwy Aqueduct	Dec. 19th	2 30 pm.	4 pm.	4	21
Windsor Well	Jan. 31st	1 30 pm.	5 pm.	14	—
Windsor Well	July 8th	11 am.	12 am.	24	13
Windsor Well	Aug. 6th	11 am.	11 40 am.	58	11
Windsor Well	Aug. 31st	3 pm.	3 30 pm.	40	15
Windsor Well	Sep. 26th	11 am.	11 15 am.	28	22
Windsor Well	Oct. 25th	12 am.	12 20 pm.	140	8
Windsor Well	Nov. 25th	9 30 am.	—	97	—

WATER—Miscellaneous Samples.

Source.	Date 1898.	Time of collecting.	Time of examination.	Bacteria present. Gelatine. per c.c.	Agar. per c.c.
Anderton Hall	Aug. 31st	4 45 pm.	9 10 am. Sep. 1st.	800	2000
Paddington	Sep. 8th	3 pm.	3 pm.	20	12
Eustace Street	Sep. 8th	3 pm.	3 pm.	136	120
Sefton Park (Croxteth Road)	Sep. 12th	—	4 pm.	20	6
Croxteth Road	Sep. 12th	—	—	26	6
Grantham Street	Sep. 15th	—	10 am.	10	28
Chatham Street	Nov. 14th	—	4 pm	50	21
1 Hope Street	Nov. 14th	—	4 pm.	70	16
16 Walnut Street	Nov. 14th	—	4 pm.	60	10
175 Brownlow Hill	Nov. 14th	—	4 pm.	44	7
58A Grove Street	Nov. 14th	—	4 pm.	30	8

CLASS III

Special Bacteriological Investigations.—Under this heading 10 examinations were made. There were 2 cases of suspected food poisoning where bacteriological examination failed to reveal the presence of pathogenic organisms. Anthrax bacilli were shown to be present in one case of spleen sent for examination.

An investigation of several samples of hay, to determine whether the bacillus anthracis was present or not, as the hay was suspected to be the cause of infection, gave negative results, as the anthrax bacillus was not found.

Autopsies.—One autopsy was performed upon a suspected case of death from cholera, and the bacteriologist was able to definitely state that death was not due to cholera but to ptomaine poisoning from eating unsound food.

MISCELLANEOUS ARTICLES

Article.	Date 1898.	Remarks.
Liver	Feb. 2nd	Normal.
Portion of Cow's Udder	Feb. 2nd	Normal.
Pork (1)	April 5th	No pathogenic organisms.
Pork (2)	April 5th	No pathogenic organisms.
Piece of Spleen	July 27th	Anthrax bacilli present.
Hay	Aug. 2nd	No anthrax bacilli found.
Hay (1)	Aug. 11th	No anthrax bacilli found.
Hay (2)	Aug. 11th	No anthrax bacilli found.
Hay (3)	Aug. 11th	No anthrax bacilli found.
Food	Oct. 31st	Normal.

CLASS IV

Examination for Rabies.—The head of the dog which is suspected to have been suffering from rabies is, by permission of the Board of Agriculture, sent to the laboratory, and a subdural inoculation of an emulsion of the brain made. The brain is very carefully examined for any signs of congestion. In more than one instance during the year the bacteriologist was able, at a very early period, to allay the naturally great cause of anxiety which the bite from a suspected rabid dog produces. Out of the 26 cases examined no signs of rabies were found.

RABIES

Date 1898.	Source.	Remarks.
Jan. 1st	Prescot Street Police Station	No rabies
Jan. 11th	Breck Road ,,	No rabies
Feb. 3rd	Rose Hill ,,	No rabies
Feb. 16th	Lark Lane ,,	No rabies
Feb. 20th	Jordan Street ,,	No rabies
Mar. 14th	Jordan Street ,,	No rabies
Mar. 14th	Kingsley Road ,,	No rabies
Mar. 29th	Rose Hill ,,	No rabies
Mar. 29th	Rose Hill ,,	No rabies
April 12th	Lark Lane ,,	No rabies
April 23rd	Olive Street ,,	No rabies
May 9th	Warren Street ,,	No rabies
June 16th	Fairfield ,,	No rabies
June 18th	Durning Road ,,	No rabies
June 28th	Seel Street ,,	No rabies
July 8th	Olive Street ,,	No rabies
July 26th	Prescot Street ,,	No rabies
Aug. 2nd	Breck Road ,,	No rabies
Aug. 6th	Westminster Road ,,	No rabies
Sept. 2nd	Rose Hill ,,	No rabies
Sept. 9th	Prescot Street ,,	No rabies
Sept. 19th	Westminster Road ,,	No rabies
Oct. 6th	Rose Hill ,,	No rabies
Oct. 28th	Essex Street ,,	No rabies
Nov. 29th	Westminster Road ,,	No rabies
Dec. 17th	Breck Road ,,	No rabies

THE BACTERIOLOGICAL INVESTIGATIONS UNDERTAKEN FOR THE WATER COMMITTEE OF THE CITY OF LIVERPOOL*

In November, 1897, arrangements were made with Professor Boyce, M.B., University College Liverpool, to undertake systematic examinations of the bacteria in the Liverpool waters, and special investigations of the sources of supply.

Report of Professor Boyce

The bacteriological analysis of water is universally regarded as furnishing us with the most delicate means of ascertaining the purity of a water supply. By its means we are enabled to state :—

1. That pathogenic bacteria, such as the organisms of typhoid and cholera, are absent.
2. That the water is free from those organisms which are found in the alimentary canal of man and animals, such as the Bacillus coli and the Bacillus enteriditis sporogenes, organisms which would strongly tend to indicate sewage contamination.
3. That the sand filters work efficiently and retain the greater proportion of the bacteria present in the unfiltered water.

The bacteriological analysis is of two kinds :—

a. Quantitative. *b.* Qualitative.

Quantitative analyses determine the number of bacteria present in a cubic centimetre of water. It is found in practice that when the number of micro-organisms is small (under 100) the chances of pathogenic organisms or organisms indicating sewage contamination being present is very remote. In order, however, to definitely determine the presence or absence of pathogenic organisms a qualitative analysis is made.

Qualitative Analyses

These are made with special media and under special conditions of incubation, in order to favour the growth of the particular organisms it is desired to find to the exclusion of other bacteria. Two organisms which are regularly sought for in this way are the Bacillus coli and the Bacillus enteriditis sporogenes. In addition to these occasional examination is made for thermophilic bacteria. This curious class of organisms grows at a very high temperature—between 50° and 70° C.—at temperatures, therefore, which readily kill ordinary putrefactive and pathogenic bacilli. From time to time various species of bacteria are isolated and cultivated and propagated in the laboratory, and it has been found that certain species are common in unfiltered and rare in filtered water. The sand of the filter bed has its special organisms, and sometimes from the dead end of pipes almost pure cultivations of one particular species can be found. The

* Reprinted from the 'Report of the Water Engineer.'

bacterial flora which is being gradually formed is of use for reference. The classification which we adopt is an artificial one, and based upon the action of bacteria upon various media.

Comparison of Results with those of other Towns

As far as we are aware, the routine bacteriological examination of the water as carried out in Liverpool is practised in no other large city in Great Britain. On the continent daily examinations are common. At first it was the practice in some places abroad to examine the water from each filter bed, in order to ascertain whether each bed was working satisfactorily. Now, however, it is usual to simply examine the water as it is supplied to the town.

The following is a comparison of the number of bacteria per c.c. present in the water supplied to a drinking fountain in the city of Liverpool and that supplied to certain other large cities in Europe :—

Liverpool	24	bacteria	per c.c.
Hamburg	70	„	„
Berlin ... ... under	50	„	„
Paris	1,125	„	„
Buda Pesth ... ... under	100	„	„
Munich	4	„	„

From these figures it will be seen that the water of Liverpool is exceptionally good, that of Munich alone being better. Further, that as neither the B. coli nor the B. enteriditis sporogenes have been found in the filtered water the purity is still further accentuated.

METHODS EMPLOYED

The following methods are used for both classes of investigation.

Collection of Samples

Small (1-ounce capacity) sterilized bottles are used for collecting the water from the mains. For collecting water at considerable depths vacuum tubes which can be broken under water are employed. Each bottle has a distinctive number ground on it, and the particulars of the samples are entered in a note-book at the time of collecting.

Time of Making the Analysis

The bacteriological investigations are made as soon after collecting as possible. In the case of the daily examinations the average time of analysis after collection is one half-hour. In the case of the fortnightly and monthly samples a delay of two to three hours occurs ; but as the sample-bottles are kept enclosed in a special ice-box during the delay of two or three hours there is very little chance of bacteriological multiplication. If in special cases there is a greater delay, the special box containing the samples is placed in the refrigerator chamber.

Media

Gelatine.—The composition of the gelatine is constant, and the reaction is standardized.

Agar.—Carbolized agar, in the proportion of 1 in 1,000, is always used.

Milk.—Fresh sterilized litmus milk tubes are used.

Temperature of Incubation

For gelatine	20° C.
For carbol agar	42° C.
For milk	37° C. (anærobically)

Time of Incubation

For gelatine	72 hours
For agar	24-48 „
For milk	24-48 „

Dilutions

In the case of all drinking waters, and in other cases where the number of organisms will probably not exceed 300 in 1 c.c., one cubic centimetre of the water is mixed with the gelatine, agar, and milk.

In those cases where the number or organisms is probably great, dilutions of 1 in 10, 1 in 50, 1 in 100, &c., are used.

Use of the Various Media

The *gelatine* is used for quantitative analysis only, serving to indicate how many bacteria are present in 1 c.c. of water. The counting is made at the end of 72 hours. The proportion of liquefiers to non-liquefiers is not taken into consideration. A collection has been formed of all the different organisms isolated from the water samples, and these are kept alive by constant re-inoculation.

The *carbol agar*, to which 1 c.c. of water is added, is for determining the presence or absence of the B. coli in that quantity of fluid. The proportion of carbolic acid in the agar is 1 in 1,000 when the 1 c.c. of water to be tested is added.

The *milk*, to which 1 c.c. of the water is added, is used for ascertaining the presence or absence of the B. enteriditis sporogenes. One cubic centimetre of the water is added to sterilized litmus milk and heated at 80° C. for 15 minutes, then incubated at 37° C. anærobically.

The bacteriological investigations which have been made since the year 1898 may be described as experimental and routine.

I. The Experimental Investigations

These consist of the quantitative and qualitative analysis of—

1. *Unfiltered Waters.*
   - (*a*) Samples of the surface moorland of the watersheds.
   - (*b*) Streams and rivulets running into reservoirs.
   - (*c*) Land drains running into reservoirs.
   - (*d*) Reservoirs.
   - (*e*) Conduits leading the water to the filter-beds.

The above examinations are conducted on the *Rivington* and *Vyrnwy* watersheds.

2. *Wells.*—Experimental investigation is made from time to time to ascertain the number of bacteria present in the bore-holes and issuing from the numerous fissures at the bottom of the well. Analyses are also made of the water at various periods under ordinary conditions of pumping.

3. *Dead Ends.*—From time to time the water in the dead ends of the mains is investigated, samples being taken at minute intervals during the 10 to 15 minutes when the pipe is being flushed.

4. *Service Reservoirs.*—These are from time to time examined. Bacterial analyses are also made to ascertain the effect of varying rates of filtration upon the filtered water; also the condition of the filtered water after passing through a newly-made bed, or bed the surface of which has been scraped.

At Oswestry the water engineer has constructed numerous small test-filters, and the effluent from these is periodically examined.

5. *Deposits and Filtering Materials.*—From time to time quantitative and qualitative analyses are made of the deposits forming in the interior of the unfiltered water-pipes; also of the sand composing the filter-beds, and of the sand at the bottom of the wells.

II. The Routine Investigations

These are—

(*a*) Daily	...	Drinking water supplied to city and collected on Mount Pleasant.
(*b*) Fortnightly		Ashton Hall tap.
(*c*) Monthly	...	The three wells—(*a*) Windsor, (*b*) Dudlow Lane, (*c*) Green Lane; the Rivington and Vyrnwy water at Prescot, and the mixed water at Prescot.

At the present time the analysis is quantitative and qualitative, and the **standard** is that the water contains—

(*a*) Less than 100 bacteria per c.c.
(*b*) No B. Coli
(*c*) No B. enteriditis sporogenes.

RESULTS OF THE ANALYSES IN 1899

Daily Analyses

Since the 4th December to 3rd February, 1900, the average number of bacteria per cubic centimetre in the drinking water supplied to the city, and obtained every morning from a public drinking fountain in Brownlow Hill, corner of Mount Pleasant, is 24 per c.c.

Fortnightly Analyses

These have been made from a tap in Ashton Hall (Museum of Hygiene) supplied directly from the main. Average number of bacteria 55 per c.c.

Monthly Analyses	Average number of bacteria per c.c.
Vyrnwy Water.—Samples taken at Prescot	38
Rivington Water.—Samples taken at Prescot	34
Dudlow Lane Well	48
Green Lane Well (excluding temporary increase through disturbance by workmen)	95
Windsor Well	57

SPECIAL INVESTIGATIONS

Rivington

The unfiltered water going on the filtered water-beds contained 100 bacteria per c.c. The average in the filtered water was 30 per c.c.

Lake Vyrnwy and Oswestry

Samples taken from various parts of the lake and from various depths gave an average number of 31 bacteria per c.c. Samples from the principal feeders of the lake gave an average of 91 bacteria per c.c. An examination of the water at several points along the course of the pipe-line, from Vyrnwy to Oswestry, showed the number of bacteria per c.c. to be at Hirnant, 42; at Parcuchaf, 28; and at Cynynion, 34.

The number of bacteria in the unfiltered water at Oswestry varied very greatly. Samples were collected from various places in the filters above the sand-beds, and it was found, as might be expected, that the number of bacteria was far greater in the corners than near the point of entry. The highest number of bacteria varied from 700 to 800 per c.c., and probably an average of 50 per c.c. would be correct. The water in the pipe-line supplying the Oswestry filter-beds contained, on an average, 40 per c.c., and it is therefore highly probable that an increase takes place in the water standing upon the filter-beds during the filtration process. The average of the filtered water as examined at Oswestry was 30 per c.c.

Well Water

During the year 1898 and 1899 opportunity was taken, when the Green Lane and Windsor Wells were emptied, to make a thorough examination of the water issuing from the numerous fissures in the side and vault of the wells, and also of the water issuing from the bore-holes.

The examination proved of very great interest. It was found that the number of bacteria varied very greatly in the water issuing from the fissures. The highest number was 800 per c.c., whilst some samples were sterile. The average for all samples for the Green Lane Well was 134 per c.c., and that for the Windsor Well 73 per c.c. The sand on the bottom of the well was subject to a bacteriological analysis and the B. enteriditis sporogenes was found.

Dead Ends

It was important to determine how far bacteria multiplied in the stagnant water at dead ends of mains. When the water in a dead end is allowed to flow out the first rush is often clear, but it is immediately followed by black and rusty water. Much to my surprise the number of bacteria present in the very black and rusty water is very small. The highest number out of the numerous series of analyses was 1,500 per c.c., and the average was about 600 per c.c.

When the water had become clear again, which usually takes place in 7 to 10 minutes, the average came down to 40 per c.c. These observations are most satisfactory.

BACTERIOLOGICAL INVESTIGATIONS FOR OTHER CORPORATE BODIES

Arrangements have recently been made with the Waterloo and Seaforth District Council, with the Urban District Council of Hindley, and with the Corporations of Warrington and Wigan, for systematic bacteriological analyses, and the report of the work done for these bodies will appear in a subsequent volume of the *Thompson Yates Laboratory Reports.*

THE PATHOLOGICAL DIAGNOSIS SOCIETY OF LIVERPOOL

W. B. WARRINGTON, M.D., DIRECTOR

INTRODUCTION

The introduction of the more exact methods of investigation to the Art of Medicine has added considerably to the ability of the practitioner to diagnose the various forms of disease.

So much is this so that a Clinical Laboratory is regarded by many as a necessary adjunct to a hospital. Important works dealing with the application of Bacteriology and Chemistry to bedside medicine have been published by VON JAKSCH, Simon of the JOHN HOPKINS University, HUTCHISON and RAINEY, and others, and have done much to emphasize the value of following cases in the Laboratory. Much of the work and time of a distinguished Liverpool alumnus, the late Professor KANTHACK, was devoted to the same idea, viz., the intimate relations which should subsist between the bedside and the Laboratory. In May, 1897, Professor BOYCE considered that an attempt should be made to place the facilities offered by the Laboratory at the disposal of the Medical Practitioners of the neighbourhood.

Invitations were accordingly sent to a large number of members of the profession residing in the North of England and Wales, asking their co-operation in the formation of a society whose object it should be to thus supplement the work of the clinician.

About 150 practitioners responded to the invitation, and promised a small annual subscription in order to defray the necessary working expenses. With the removal of the Pathological Department to the THOMPSON YATES Laboratories the labour of those who undertook the examination of the various morbid specimens was greatly faciliated, and permitted of a more satisfactory organization. The society is now nearing the close of its third year, and the number of reports issued to its members has doubled since its foundation. There can be little doubt that the value of work of this kind will increase, and it is within very recent date that the important agglutinative action of Typhoid serum has been utilised in a routine way for the diagnosis of that insidious disease.

WORK OF THE SOCIETY

1. Medical Practitioners joining the society are enabled to receive, free of cost, reports on the following subjects :—
    1. Suspected tuberculosis.
    2. Suspected diphtheria.
    3. Typhoid serum reaction.
    4. Nature of tumours.
    5. Nature of urinary deposits.

More extensive investigations can, however, be undertaken should occasion arise.

2. Specimens from Hospitals will also be investigated. Reports are at present issued to
   The Children's Infirmary, Liverpool.
   The Royal Albert and Edward Infirmary, Wigan.
   The Southport Infirmary.
   The Asylum, Chester.
   The Wirral Children's Hospital.
3. Members are provided with appliances for forwarding the specimens.
4. Reports are issued as early as possible.

DIRECTIONS FOR SENDING MATERIAL

Sputum

A small quantity only is necessary, and it is advisable that the more solid portion of the expectorated material should be sent, unmixed with any fluid. For transmission, a clean wide-mouthed bottle is best. Marked hæmorrhagic sputum is unsatisfactory for examination.

Diphtheria

A sterilized swab is provided.

The directions are as follows :—

Before using any antiseptic application to the throat, rub the swab on the diseased surface, endeavouring to obtain as much material as possible ; the minimum should be at least visible to the naked eye ; return to the test tube and post *at once* to the Laboratory.

In suspected laryngeal diphtheria the material should be obtained as far back in the pharynx as possible.

Typhoid Serum

Capillary tubes for collecting blood are provided.

Members should bear in mind the following points :—

1. The reaction is not often obtained till after the first week of illness, hence, in all early cases in which the report is negative, a second sample of blood should be sent.
2. Before collecting the blood, the skin should be thoroughly washed and cleansed in spirit and then allowed to dry. The lobe of the ear is a very convenient place from which to obtain the blood.

Blood

i. *Examination for organisms.*—The use of the several antitoxic sera renders a preliminary examination of the blood necessary. Statistics show that organisms are found in about 10-20 of suspected cases.

It is advisable to send as large a quantity of blood as possible, and this is best done by withdrawing a syringeful from a superficial vein of the arms, strict antiseptic precautions being taken.

ii. *Microscopic examination.*—Thin films should be made on perfectly clean coverslips at the bedside and forwarded to the Laboratory.

Tumours and other Tissues for Histological Examination

In case of tumours, select from the edge of the new growth, and, if possible, include some of the adjacent healthy structure. Those parts of a tumour showing caseous or degenerative change should be avoided.

As the nature of a tumour is often a question of the utmost importance, we must emphasize that it is not always in the power of the pathologist to give a positive answer as to the nature of the growth. In such doubtful cases the clinical characteristics are more important than the histological features.

Method of sending material.—The most convenient way is to wrap the piece of tissue selected for examination in cotton wool moistened with weak spirit or 4 °/₀ formol.

W. B. Warrington, *Director.*

MUSEUM REPORT, 1899

During the past twelve months over 100 new preparations have been added to the museum. In many cases the specimens were of great value in illustrating pathological conditions of some rarity.

We may note among the specimens related to the Alimentary Tract—

A 8 x. Epithelial pearls from a median dermoid of the floor of the mouth.

A 121 x 2. Tubercular ulcer of the stomach.

A 190 x. Stricture of the Jejunum, the sequel of a simple ulcer.

B 10 x 1. Abscess of the liver, rupture into pericardium.

B 36 x 1. Adenoma of the liver, with a secondary green growth in the mediastinum.

B 75 x. Hæmorrhagic pancreatitis.

D 78 x. Carcinoma of the kidney, originating in the glomeruli.

In the gynecological section many important specimens have been added by Professor BRIGGS, and Dr. WILLETT has given valuable assistance in preparing them for the museum.

In the section devoted to diseases of the circulatory system we may single out—

I 12 x 1. Calcareous pericardium.

I 22 x. Pigmented heart in ADDISON's disease.

I 75 x. Tubercular endocarditis.

I 161 x. Dissection of the neck after ligation of all the carotid arteries, and all the jugular veins on the left side.

In the nervous section a few specimens have been added, but diseases of this system are perhaps the most poorly represented section of our museum.

A specimen of the black variety of Madura foot has been added; this was due to Captain BENNETT, I.M.S., an old student, and we hope that many of our men who may come across specimens will not forget us.

A very important addition to the museum in the form of a large series of **radiographs** (200), we owe to Dr. THURSTAN HOLLAND, and it is hoped that sufficient money will be found to have them suitably mounted and placed in the museum for permanent reference.

COMPARATIVE PATHOLOGY REPORT

By J. F. RYDER

During the past year special efforts have been made to increase the number of Animal Pathological specimens in the museum, and to make this department of Comparative Pathology more complete; to add specimens showing the close relationship existing between the diseases of man and those of the lower animals; and to make the collection not only one of scientific interest but of practical value to students of research, D. P. H. students, and meat inspectors.

The museum now contains about 100 specimens, and new specimens are being received and mounted almost daily. In many cases the specimens are of great value in illustrating pathological conditions in different animals. In the majority of specimens sections have been cut and mounted for microscopical examination and can be seen at any time.

We may note among the specimens such interesting ones as—

No. 44. Actinomycosis of tongue. Bovine.

No. 49. Œosophagostoma columbianum, or nodular disease of intestines in sheep.

No. 52. Tubercular mammary gland from cow. Animal giving milk.

No. 64. Tubercular lungs, liver, and intestines from a fowl. Avian tuberculosis.

No. 66. Tubercular lungs and liver from a goat. Very rare.

No. 67. Tubercular lungs from calf two weeks old.

No. 69. Carcinoma of mamma, bitch. Secondary growths in liver, spleen, lungs, and on pleura.

No. 74. Tubercular spleen. Bovine.

No. 80. Liver from pig, showing numerous gall-stones from size of pea to hen's egg.

No. 16. Nasal septum from horse. Glanders showing ulcers.

A descriptive catalogue of the museum will be ready very shortly.

REPORT OF THE HOLT FELLOW IN PATHOLOGY

E. E. GLYNN, B.A. CANTAB., M.R.C.S.

The Fellowship was founded for the promotion of Pathological Research, and in order to give the Professor of Pathology an Assistant in the work of the Department. The appointment is an annual one, and has produced great good. Dr. CHRISTOPHERS, at present on the Royal Society's Malarial Expedition, Dr. ANNETT, and Dr. DUTTON, engaged upon the second Liverpool West African Expedition for the Study of Malaria, have all been Holt Fellows.

The work of the Fellow has consisted in—

(*a*) Preparing microscopical specimens for the students' classes in morbid histology and bacteriology.

(*b*) Making microscopical and bacteriological examinations for the Professor of Surgery.

(*c*) Conducting certain investigations on *Bacillus coli* and *Bacillus enteritidis sporogenes*.

THE COLONIAL FELLOWSHIP

This Fellowship has been most generously founded by Mr. WILLIAM JOHNSTON to encourage Bacteriological Research. It is open to graduates of our Colonial Universities, in order to afford them an opportunity of coming to Europe and of seeing what is being done in other places. It will, no doubt, conduce to further increase the bond of union which exists between this Country and her Colonies. The Fellowship is vacant at present.

REPORT OF THE ALEXANDER FELLOW

A. STANLEY GRIFFITH, M.B.

The ALEXANDER Fellowship was founded by Dr. ALEXANDER, in July, 1899, and Dr. A. STANLEY GRIFFITH appointed the first Fellow. The Fellowship was founded to promote the advancement of Medicine by Research. The main object of the Fellowship is research; but the Fellowship carries with it certain duties in connection with the BROWNLOW HILL WORKHOUSE INFIRMARY, and the MAGHULL HOME FOR EPILEPTICS, such as the examination of tumours, blood, and bacteriological analysis. The material furnished from both these institutions, numbering some 2,000 patients, offers a very large field for investigation. The ALEXANDER Fellow has already taken advantage of his facilities, and has commenced an investigation into the bacteriology of the various forms of Ophthalmia. A preliminary report of this investigation is appended.

PRELIMINARY REPORT ON OPHTHALMIA

Histological Appearance of an Acutely Inflamed Conjunctiva

The areolar tissue beneath the epithelium and the tissue around the meibomian glands is infiltrated with numbers of small round cells; the infiltration is general, but here and there the cells are aggregated in small masses; the masses are distinctly circumscribed, yet do not possess any capsule. The epithelial layer of the conjunctiva has suffered some loss, especially at those places adjacent to the round cell collections. The vessels are dilated and engorged with blood. Numerous coarsely granular plasma cells are observed, some of which have been ruptured with a discharge of their contents into the surrounding tissues. A few bacilli were detected, but only in small numbers.

During the investigations I was fortunate enough to obtain some poorly nourished kittens, suffering from muco-purulent ophthalmia, the eye discharge of which contained a bacillus somewhat similar to that present in muco-purulent ophthalmia. Although the leucocytes of the discharge contained the bacillus in appreciable numbers, the vast majority were discovered massed together around and in close association with epithelial cells. The bacilli occurred mainly in pairs, and varied little in form; the longer bacilli had squarish ends with a very small interval between the elements; shorter forms were oval and had a distinct interval. The tissues of the conjunctiva were infiltrated with small round cells which were here and there grouped together in small masses; the vessels were engorged with blood, and many plasma cells were present.

It may be of some interest in this connection to describe the histological appearances of a kitten's cornea, rendered opaque by a severe acute inflammation.

At the periphery of the cornea there is the normal stratified layer of epithelium, consisting of columnar epithelium on a basement membrane, with cubical and squamous cells above. As the opacity is approached the thickness of the epithelium diminishes; first it is represented by a double layer of cells, then a single layer of cubical cells, and finally, when the opacity is reached, the epithelium has entirely disappeared.

Where the epithelium has disappeared the basement membrane cannot be detected, and the substantia propria cornæ for a thickness of 6 or 7 laminæ has undergone a kind of coagulative necrosis; it is represented by an open network of slender fibres enclosing some degenerated remnants. In several places the continuity of this network is broken by a further destruction of tissue in the shape of a minute ulcer. The edges are fairly smooth, and the floor is only separated from the normal substantia by one or two necrosed lamallæ.

At the periphery of the cornea, and for a short distance into its substance, there is a round cell infiltration, with deposits of blood-pigment in the dilated blood-vessels and the spaces of the corneal substance.

Histology of Inflamed Conjunctiva in which there are a few Granules

There is the same abundant leucocytic infiltration as in the acutely inflamed conjunctiva; but the small round cells are chiefly aggregated in the tissues immediately beneath the epithelium, and around the Meibomian glands; the areolar tissue is not so profusely infiltrated. At this stage a more distinct formation of the round cell masses is observed beneath the epithelium and in the loose subepithelial tissue. The masses are quite isolated, but do not possess any distinct capsule; running around the cells, however, is a delicate reticulum of fibrous tissue. When the mass is situated beneath the epithelium it causes on the surface of the conjunctiva a projection, which consists of dense, deeply-staining fibrous tissue almost entirely denuded of epithelium, one or two columnar cells alone remaining. In one specimen this thickening at the conjunctival surface had not occurred and the mass of cells had apparently ruptured, the contents being partly discharged. Very few plasma cells could be observed. There was early formation of young connective tissue in some amount.

Section through a Typical Granuloma

Granules consist of small round cells enclosed by an incomplete capsule of bands of fibrous tissue, and a delicate reticulum of fibrous tissue around the cells. Bands of connective tissue also run through the mass. Around the granule there is very little leucocytic infiltration, and plasma cells are not so abundant. Slender bacilli were found in large amount in one instance.

Examination of the Discharge from different cases of Ophthalmia

Muco-Purulent Ophthalmia

Method of Examination.—After the lid had been completely everted some of the discharge was taken from the fornix conjunctivæ on the loop of a platinum wire, and cover-glass preparations were made in the usual way. By this method a sample of pus was obtained free from the risks of contamination incurred if taken from the margins of the lids, or from the internal canthus. The pus consisted of large numbers of poly- and uni-nuclear leucocytes, epithelium cells, strings of fibrin, and bacteria. The bacteria found are of several varieties, the one of most frequent occurrence was a short, slender bacillus, usually seen in pairs; but also found singly and in short chains. These bacilli were seen not only outside the leucocytes but also massed together inside, and often entirely filling them; other leucocytes had a smaller number, and some contained only one or two in their substance; they were also seen in close association with epithelial cells. The bacilli were only found in leucocytes when the reaction of the tissues was great. In the more chronic cases the leucocytes did not contain them

The bacillus occurred principally in a diplo-form, although it was often seen singly and in short chains. Its numbers were sometimes enormous, especially in the acuter forms of muco-purulent catarrh; the numbers diminished as the acute symptoms subsided. A morphologically similar organism has been detected in normal nasal mucus.

Granular Conjunctivitis

When the discharge is scanty isolated examples of the slender bacillus are seen. Cocci, diplococci, and xerosis bacilli occur in small numbers.

Acute discharge from trachomatous eyelids contains quantities of the slender bacillus; they are also found enclosed in leucocytes.

The other organisms are not increased to any great extent.

Acute Ophthalmia

A few cases have been examined. Whereas in muco-purulent ophthalmia the slender bacillus is invariably the causal agent, acute ophthalmia may be caused by different organisms. The gonococcus was chiefly the cause of acute conjunctivitis in infants. In older children and adults the pneumococcus was often found to be the cause of an acute ophthalmia. The Koch-Week's bacillus can excite an inflammation indistinguishable from gonorrhœal inflammation.

The organisms found in and cultivated from the eye discharges were of a numerous and varied description.

All the common varieties of staphylococci were found. Staphylococcus aureus occurred most frequently, and was invariably found when there was any blepharitis.

Staphylococcus albus occurred next in frequency, and was especially found in cases of trachoma.

Biscuit-shaped cocci were cultivated in many instances; they probably represent the trachom-cocci of Sattler.

Pure cultures of the zerosis bacillus were obtained in about 80 % of all the inflamed eyes examined; it was in a large number of cases the only organism cultivated. It grew most readily on serum, and often liquefied this medium in the form of little pits.

The *bacillus lacunatus*, described by Eyre, was found to have a strongly liquefying action on serum: the liquefied serum contained grey and white masses, and was turbid with suspended growth. Microscopically, the cultures in the first instance were impure containing cocci, slender bacilli, and the *bacillus lacunatus*. This bacillus mainly occurred in pairs and measured 1.5 μ by 2 μ in length by 1 μ in breadth.

A colon bacillus was cultivated in 8 or 10 cases. It occurred in simple conjunctivitis, muco-purulent ophthalmia, and dacryo-cystitis.

Cultivation on artificial media of the slender bacillus found in the discharge of muco-purulent catarrh and acute trachoma was very difficult. It does not grow on the ordinary laboratory media. A limited growth has, however, been obtained on human blood agar and on serum. The growth, in one instance, consisted of a number of minute colonies aggregated together to form a small transparent irregular mass. Single colonies were small and circular, the margins slightly raised above the surface of the medium, and the centre rounded and elevated. Older colonies have a frilled margin. The bacillus was commonly found growing in the neighbourhood of xerosis or staphylococcus colonies. In cultures the bacillus occurs singly, in pairs, and in threads. There is usually a distinct interval between the elements, especially when the individuals forming a pair have a short oval shape. The longer bacilli form palisades.

Staining reactions.—It stains well with fuschin, and poorly with methylene blue and dahlia; the stain is not retained by Gram's method.

Thermal death point.—About 60° C., exposed for 10 minutes.

Anäerobiosis.—It is an obligate anäerobe.

Clinically it is impossible to differentiate the various diseases manifesting themselves in the acute affections of the conjunctiva. A correct diagnosis can only be arrived at with the aid of the microscope. By examination of numerous acute discharges it is certain that the bacillus first discovered by KOCH, and afterwards more fully described by WEEKS, is capable of exciting quite as acute an inflammation as the gonococcus. In many cases in which the KOCH-WEEK's bacillus was the only organism discoverable the eye condition closely resembled that of gonorrhœal ophthalmia. The quality and quantity of the discharge and the severity of the inflammation are not, therefore, certain guides to a diagnosis of acute inflammations of the eye. Acute inflammations of the eye are, undoubtedly, caused in many cases by gonococcus infection ; but a diplococcus, exactly resembling gonococcus, has been found in acute discharges and enclosed in leucocytes, which stains by GRAM's method.

The causal agent of muco-purulent catarrh is almost invariably the KOCH-WEEK's bacillus; the discharge in the more acute cases showed great numbers of the bacillus enclosed in leucocytes. With a decrease in the severity of the symptoms the number of leucocytes containing the bacillus becomes less.

In a true case of granular lids with a very small amount of discharge there are few organisms of any kind present. When a case of trachoma suddenly develops acute symptoms, the KOCH-WEEK's bacillus is found in large numbers and enclosed in the leucocytes of the discharge. The organisms that mainly occur, such as staphylococci, xerosis bacillus, &c., when inoculated in healthy human eyes do not seem capable of exciting any morbid process. The sudden development of acute symptoms, and the presence of the KOCH-WEEK's bacillus in large numbers in the discharge, is strong evidence that the acute symptoms are due to the virulent action of this bacillus. This development may be due to a separate infection, or to an access of virulence in an already existing microbe. That the microbe does exist in granular lids with very scanty discharge has been proved by the microscope. The theory of separate infection must not be disregarded, since the conditions for the development of muco-purulent catarrh are distinctly more favourable in a lid disorganised by granulations than in a previously normal one.

The well-known infectivity of trachoma, and its occurrence in crowded communities, suggest that it is due to some irritant easily conveyed from person to person; this irritant must be some bacterial organism.

Authorities differ as to the presence of microbes in the granules. SATTLER and MICHEL obtained from the contents of a granule a biscuit-shaped coccus, occurring in pairs, to which they ascribed the granular condition ; KARTULIS failed to demonstrate the presence of any organisms ; SHONGOLOWICZ in 26 cases of trachoma (? discharge) found a short bacillus not previously described. In specimens obtained from the Workhouse Infirmary a slender bacillus has been observed in the tissues.

A granule is a chronic inflammatory nodule consisting of small round cells and delicate fibrous tissue ; it ultimately becomes converted entirely into cicatricial tissue. The granular

condition may be due to the entrance into the tissues of a microbe with a low degree of virulence exciting an inflammatory process which culminates in the formation of the round cell mass; one may say that, instead of exerting an irritating influence, it has a stimulating effect on the tissues in its sphere of action.

The result is exemplified in those instances where half a school is attacked by this dire disease without any symptom occurring to indicate that any person has contracted the complaint.

Conclusions.—That acute ophthalmia has not one specific microbe.

That the short, slender bacillus is the cause of muco-purulent catarrh.

That other organisms, after the catarrh has developed, may have some action in continuing and intensifying the inflammation.

That a morphologically similar bacillus having been discovered in the healthy nasal mucus, the nose very probably supplies a primary source of infection in a conjunctiva, the resistance of which to morbid irritants has previously been lowered.

That in granular conjunctivitis no organism prevails to which the morbid condition can be assigned.

That when acute symptoms develop in a granular lid the increased inflammation is due to the short, slender bacillus.

That granules are not merely lymphoid collections but inflammatory nodules resulting from the entrance of an organism.

That clinically and microscopically there is strong evidence that muco-purulent catarrh and granular conjunctivitis may be due to the same organism acting differently with different degrees of virulence, and, possibly, with varying resistance of the tissues.

THE LIVERPOOL SCHOOL OF TROPICAL MEDICINE

REPORT FOR 1899

By A. H. MILNE

The establishment of a School in Liverpool, in connection with University College and the Royal Southern Hospital, for the special study of Tropical Diseases, was originally suggested at the Annual Dinner of the Royal Southern Hospital, held on 12th November, 1898. The suggestion met with approval, and steps were immediately taken for the formation of such a School.

COMMITTEE

A Committee, representative of the various interests concerned, was formed under the Chairmanship of Mr. Alfred L. Jones, the originator of the proposal. Mr. William Adamson, President of the Royal Southern Hospital, was appointed Vice-Chairman (representing together with Mr. A. L. Jones the Committee of the Royal Southern Hospital), and the following gentlemen were nominated by the public bodies named:—Principal Glazebrook and Mr. Charles W. Jones, by the Council of University College; Professor R. W. Boyce and Professor A. M. Paterson, by the Senate of University College; Professor Carter and Dr. Alexander, by the Medical Board of the Royal Southern Hospital; Mr. J. O. Strafford and Dr. E. Adam, by the Chamber of Commerce; Mr. C. Booth, Jr., and Mr. J. W. Hughes, by the Liverpool Steam Ship Owners' Association, and Colonel Goffey and Mr. J. Chadwick, by the Liverpool Shipowners' Association. Professor Boyce was elected Dean of the School, Mr. J. O. Strafford, Honorary Treasurer, and Mr. A. H. Milne, Honorary Secretary.

FINANCE

A subscription list was opened, headed by a yearly contribution of £350 promised by the Chairman for 3 years. A gratifying response was made to the appeal, not only in Great Britain, but abroad, and the total amount subscribed in 1899, together with fees and other items, amounted to slightly over £3,000. A list of the subscriptions for 1899 is appended to this Report. At the same time the expenditure has been necessarily very heavy, and with the enlargement of the School is bound to increase, but it is hoped that the scope of the School will not have to be limited owing to lack of financial support.

STAFF

The School have been fortunate in securing the services of Major Ross, D.P.H., &c., late I.M.S., as their Lecturer in Tropical Diseases, and of Dr. H. E. Annett, D.P.H., &c., as

Demonstrator. Major Ross entered the Indian Medical Service in 1881. In 1889 he studied Bacteriology under KLEIN, and obtained the Diploma of Public Health of the United Colleges of Physicians and Surgeons, England. In 1895 he was awarded the Triennial PARKES' Memorial Gold Medal and Prize for a Memoir on Malaria. In the same year Major Ross was placed on special duty by the Government of India to report on and enforce Sanitary measures in the large station of Bangalore, and in 1898 was again placed on special duty by the Government of India to investigate Malaria and also Kala-azar. He was also Staff Surgeon and Officiating Residency Surgeon at Bangalore. The chief point of interest, however, in connection with the appointment of Major Ross, is the fact that in 1897 and 1898 he, in conjunction with Dr. PATRICK MANSON, found a solution of the malaria problem, which has since been confirmed and accepted by the leading authorities.

In addition to the above-named gentlemen, the services of the members of the Medical and Surgical Staff of the Royal Southern Hospital, and of various members of the Medical Faculty of University College, have been placed at the disposal of the School.

INAUGURATION OF THE SCHOOL

The special ward set apart at the Royal Southern Hospital for the use of the School was formally opened on April 22nd, 1899, by Lord LISTER. A Public Banquet was given in the evening to Lord LISTER, to commemorate the inauguration of the School, at which many distinguished guests were present.

WORK OF THE SCHOOL

During the first part of the year the work consisted chiefly in organizing the material at the disposal of the School, and the fitting and furnishing of the laboratories.

A Swedish missionary took out the course for missionaries during this period, and a medical man was engaged in research work for a few weeks; further, a delegate from the Government of Belgium observed the work progress of the School during this time in order to report to the Minister of the Educational Department of the country.

On the return of the Expedition (see subsequent paragraph) the School reopened on October 11th for the Autumn Term, which closed on December 16th.

Several students took out the full course during this term; they included Professor RIBBING, Professor of Therapeutics at Lund; M. LEMAIRE, Assistant to Professor BLANCHARD, Paris; Dr. HAYWARD; Dr. PICKELS, Assistant Colonial Medical Officer, Lagos; Dr. NORRIS, who proceeds at once to the hospital at Blantyre, Central Africa, and others. A missionary took out this full course before setting out for the Niger Protectorate. Major BLACK and the Belgian delegate also attended some of the Lectures and Demonstrations.

The work for the terms consisted of—

(1) Lecture delivered three, four, and sometimes five times each week by the Lecturer on Tropical Medicine, illustrated by lantern and microscopical demonstrations.

(2) Clinical instruction in cases of Tropical Disease at the special ward of the Southern Hospital, twice or three times a week by the Honorary Physicians of the Hospital.

(3) Instructions in special clinical methods in connection with Tropical Diseases by the Lecturer and Demonstrator of Tropical Medicine.

(4) Practical work and demonstrations on the parasitology, bacteriology, and pathology of Tropical Diseases by the Demonstrator at the THOMPSON YATES Laboratory, University College.

The School is indebted for material and specimens for teaching and museum purposes to Dr. PATRICK MANSON, Dr. THIN, Professor BLANCHARD, and to medical men in India, Cape Colony, and West Africa, including the Royal Society's Malaria Commission.

The Committee have issued during this term two special memoirs, written by members of the teaching staff of the School—

No. I. Instructions for the Prevention of Malaria, intended for persons residing in malarious places.

No. II. Report of the Malaria Expedition to W. Africa.

HOSPITAL REPORT

There has been an abundance of cases of Tropical Disease treated in the special ward of the Royal Southern Hospital during the year under revision, as the Table annexed to this Report shows. From this Table it appears that 176 cases in all were treated, of which 152 were Malarial Fever cases, 2 Malarial Neuritis, 3 Blackwater Fever, 5 Dysentery, 3 Sprue, 1 Diarrhœa, 1 Malta Fever, 5 Beri-beri, 2 Hepatic Abscess, 1 Scurvy, and 1 Bilharzia Disease; out of all these only 6 patients died. The nationalities of the patients were as follows:—English 151, Norwegian 5, German 3, Finnish 3, Swedish 2, Lascar 3, Italian 2, Chinese 2, and 1 American, Dutch, Spanish, Malay, and Austrian. The interesting points in connection with this Table are (1) the variety of diseases treated, and (2) the different nationalities of the patients. With more accommodation the list will, no doubt, be largely extended, but as it stands it thoroughly justifies the establishment of a Tropical Diseases School in Liverpool.

EXPEDITION TO WEST AFRICA

The most important result, however, achieved by the School was the despatch of an Expedition to West Africa to investigate the prevalence there of Tropical Malaria and other diseases. The Expedition, which started at the end of July and returned in October, chose Sierra Leone as the field of their labours, owing to its proximity to Liverpool, the time at their disposal being short. The following composed the Expedition: Major Ross, Dr. H. E. ANNETT, Dr. R. FIELDING-OULD, Mr. E. E. AUSTEN (officially appointed as Naturalist by the British Museum), and Dr. VAN NECK (Delegate of the Belgian Government to the School). The United States Government would have appointed a Delegate to join the Expedition, but the short notice given, which was unavoidable, unfortunately prevented their participation. The results of the Expedition have been in the highest degree satisfactory, and a valuable Report on its labours is now in course of preparation and will shortly be issued. It may be stated that Dr. R. FIELDING-OULD also proceeded to the Gold Coast and Lagos, and his observations thereon will be incorporated in the full report of the Expedition.

The Expedition brought back a considerable amount of very valuable material for teaching purposes, more especially a unique collection of malaria infected mosquitoes, which have proved exceedingly useful for demonstration purposes.

The thanks of the School are due to the Chairman, who generously defrayed the entire cost of the passages of the Members of the Expedition, and to the British Medical Association, who voted a large sum towards the expenses of the undertaking.

TRAINING OF NURSES

The important question of specially training Nurses for the tropics has not been overlooked, and the School has already received, and trained at its own expense, in the SAMUEL HENRY THOMPSON Ward of the Royal Southern Hospital, two Nurses sent to Liverpool by the Colonial Nursing Association. This is a branch of the School that may be extended with great advantage.

RECOGNITION BY THE GOVERNMENT

At the time of the issue of this *resumé* full recognition by the Government has not yet been extended to the School, and until that recognition is given the class of students most desired, namely, medical officers about to enter into the service of the Government in Tropical Colonies, will not be attracted. It is confidently expected, however, that full recognition will shortly be given, and that the medical officers in question will be allowed the option of undergoing their course of instruction in Tropical Medicine at Liverpool. The School is indebted to the various Government Departments for many important Medical and Statistical Blue-books, and to the Colonial Office for cordial support, especially in connection with the Expedition.

In conclusion, the Committee take this opportunity of expressing their thanks to the Public and the Press for much valuable assistance; and at the same time they desire especially to thank the Members of the Expedition referred to above, who gratuitously undertook a laborious task attended with a very considerable amount of personal risk.

BIO, EXCHANGE BUILDINGS,
LIVERPOOL, *December 30th*, 1899.

APPENDIX No. 1

HOSPITAL REPORT—SUMMARY OF CASES

Month.	No. of Cases.	Malarial Fever.	Malarial neuritis.	Blackwater Fever.	Dysentery.	Sprue.	Diarrhœa.	Malta Fever.	Beri-beri.	Hepatic Abscess.	Scurvy.	Bilharzia Disease.
January	5	4	0	0	0	1	0	0	0	0	0	0
February	5	4	0	1	0	0	0	0	0	0	0	0
March	15	14	0	0	0	0	0	0	1	0	0	0
April	5	3	1	0	1	0	0	0	0	0	0	0
May	10	8	0	0	1	0	0	0	1	0	0	0
June	14	13	0	0	0	0	0	1	0	0	0	0
July	25	19	0	1	2	0	0	0	1	1	0	1
August	35	32	1	0	0	2	0	0	0	0	0	0
September	17	16	0	0	0	0	0	0	0	1	0	0
October	19	17	0	0	0	0	1	0	0	0	1	0
November	13	10	0	1	1	0	0	0	1	0	0	0
December	13	12	0	0	0	0	0	0	1	0	0	0
Total	176	152	2	3	5	3	1	1	5	2	1	1

DETAILED SUMMARY OF CASES

JANUARY

Sex.	Occupation.	Nationality.	Period as In-Patient.	Result.	Disease.
M	Seaman	English	20 days	Recovered	Malarial Fever.
M	Fireman	„	26 „	„	„
M	Boilermaker	„	16 „	„	„
M	Steward	„	13 „	„	„
M	Fireman	Malay	25 „	Death	Sprue.

FEBRUARY

Sex.	Occupation.	Nationality.	Period as In-Patient.	Result.	Disease.
M	Ship Cook	Austrian	6 days	Recovered	Sprue.
M	Steward	English	6 „	„	„
M	Seaman	„	11 „	„	„
M	Captain (Army)	„	5 „	Death	Blackwater Fever.
M	Fireman	Lascar	16 „	Recovered	Malarial Fever.

MARCH

Sex.	Occupation.	Nationality.	Period as In-Patient.	Result.	Disease.
M	Seaman	English	12 days	„	Malarial Fever.
M	Trader	„	68 „	„	„
M	Seaman	Norwegian	52 „	„	„
M	Steward	English	30 „	„	„
M	Seaman	„	12 „	„	„

MARCH—*continued.*

Sex.	Occupation.	Nationality.	Period as In-Patient.	Result.	Disease.
M	Labourer	English ...	47 days...	Recovered...	Malarial Fever.
M	Seaman...	Swedish ...		Not reported	,,
M	Steward	English ...	10 ,, ...	Recovered...	,,
M	Fireman	,, ...		Not reported	,,
M	Seaman...	,, ...	45 ,, ...	Recovered...	,,
M	Engineer	,, ...	8 ,, ...	,, ...	,,
M	Seaman...	Chinese ...	80 ,, ...	,, ...	Beri-beri.
M	Cook	German ...	8 ,, ...	,, ...	Malarial Fever.
M	Carpenter	Finn	5 ,, ...	,, ...	,,
M	Fireman	English ...	13 ,, ...	(Irregular)...	,,
		APRIL			
M	Missionary	American ...	176 days...	Recovered...	Malarial neuritis.
M	—	English ...	29 ,, ...	,, ...	Dysentery.
M	Seaman...	,, ...	5 ,, ...	,, ...	Malarial Fever.
M	Fireman	,, ...	8 ,, ...	,, ...	,,
M	Steward	,, ...	60 ,, ...	,, ...	,,
		MAY			
M	Engineer	English ...	15 days...	Recovered...	Malarial Fever.
M	Missionary	,, ...	3 ,, ...	Death ...	,,
M	Steward	,, (India)	13 ,, ...	Recovered...	Beri-beri.
M	Seaman...	,, ...	14 ,, ...	,, ...	Malarial Fever.
M	Fireman	,, ...	8 ,, ...	,, ...	,,
M	Seaman...	,, ...	7 ,, ...	,, ...	,,
M	—	,, ...	16 ,, ...	,, ...	Dysentery.
M	Station Master (Uganda)	Swede ...	14 ,, ...	,, ...	Malarial Fever.
M	Steward	English ...	24 ,, ...	,, ...	,,
M	Seaman...	,, ...	23 ,, ...	,, ...	,,
		JUNE			
M	Fireman	Lascar ...	43 days...	Recovered...	Malaria & Parotitis.
M	Seaman...	English ...	9 ,, ...	,, ...	Malarial Fever.
M	Clerk	,, ...	101 ,, ...	,, ...	,,
M	Engineer	,, ...	8 ,, ...	,, ...	,,
M	Seaman...	,, ...	83 ,, ...	Relieved ...	,,
M	Steward...	,, ...	22 ,, ...	Recovered...	,,
M	Seaman...	,, ...	29 ,, ...	,, ...	Malta Fever.
M	Steward...	,, ...	24 ,, ...	,, ...	Malarial Fever.
M	Seaman...	,, ...	47 ,, ...	,, ...	,,
M	,, ...	,, ...	12 ,, ...	,, ...	,,
M	,, ...	,, ...	25 ,, ...	,, ...	,,
M	,, ...	,, ...	10 ,, ...	,, ...	,,
M	,, ...	,, ...	21 ,, ...	,, ...	,,
M	Trimmer	,, ...	9 ,, ...	,, ...	,,
		JULY			
M	Steward...	English ...	19 days...	Recovered...	Malarial Fever.
M	Seaman...	,, ...	Not reported	,, ...	,,
M	Mariner	,, ...	13 days...	,, ...	,,
M	Steward...	,, ...	62 ,, ...	,, ...	Malaria & Pleurisy.

JULY—*continued.*

Sex.	Occupation.	Nationality.	Period as In-Patient.	Result.	Disease.
M	Seaman	English ...	14 days..	Recovered...	Malarial Fever.
M	Seaman	„ ...	16 „ ...	„ ...	„
M	Army Lieut.	„ ...	— — ...	— ...	Beri-beri
M	Seaman	„ ...	10 „ ...	„ ...	Malarial Fever
M	Cook	German ...	9 „ ...	„ ...	„
M	Fireman	English ...	40 „ ...	„ ...	„
M	Seaman	„ ...	7 „ ...	„ ...	„
M	„	„ ...	20 „ ...	„ ...	Dysentery.
M	Cyanider	„ ...	156 „ ...	Relieved ...	Bilharzia Disease.
M	Seaman	„ ...	11 „ ...	Recovered...	Malaria & Dysentery
M	Trimmer	„ ...	12 „ ...	„ ...	Malaria
Female	—	„ ...	13 „ ...	„ ...	Blackwater Fever.
M	Trader	„ ...	4 „ ...	Death ...	Dysentery.
M	Ship's Officer ...	„ ...	19 „ ...	Recovered...	Malarial Fever.
M	Seaman	„ ...	9 „ ...	„ ...	„
M	„	„ ...	7 „ ...	„ ...	„
M	„	„ ...	7 „ ...	„ ...	„
M	Clerk	„ ...	27 „ ...	Death ...	Hepatic Abscess.
M	Seaman	„ ...	15 „ ...	Recovered...	Malarial Fever.
M	„	Finn ...	9 „ ...	„ ...	„
M	„	German ...	15 „ ...	„ ...	„

AUGUST

M	Seaman	English ...	17 days...	Recovered...	Malarial Fever.
M	„	„ ...	18 „ ...	„ ...	„
M	Pantryman	„ ...	9 „ ...	„ ...	„
M	Seaman	„ ...	13 „ ...	„ ...	„
M	„	„ ...	12 „ ...	„ ...	„
M	„	„ ...	17 „ ...	„ ...	„
M	„	„ ...	12 „ ...	„ ...	„
M	„	„ ...	26 „ ...	„ ...	„
M	Fireman	„ ...	5 „ ...	Relieved ...	Malarial Anæmia.
M	Storekeeper	„ ...	19 „ ...	„ ...	Malarial Fever.
M	Seaman	„ ...	5 „ ...	Recovered...	„
M	Steward	„ ...	10 „ ...	„ ...	„
M	„	„ ...	6 „ ...	„ ...	„
M	Ship's Officer ...	„ ...	6 „ ...	„ ...	„
M	Fireman	„ ...	10 „ ...	„ ...	„
M	„	„ ...	10 „ ...	„ ...	„
M	Sailor	„ ...	14 „ ...	„ ...	„
M	Ship's Officer ...	„ ...	6 „ ...	„ ...	„
M	Fireman	„ ..	10 „ ...	„ ...	„
M	Steward	„ ...	13 „ ...	„ ...	„
Female	Married	„ ...	28 „ ...	Relieved ...	Sprue.
Female	Nil	„ ...	28 „ ...	Recovered...	„
M	Seaman	„ ...	15 „ ...	„ ...	Malarial Fever.
M	Labourer	„ ...	10 „ ...	„ ...	Malarial Neuritis.
M	Seaman	„ ...	16 „ ...	Relieved ...	Malarial Cachexia.
M	Ship's Officer ...	„ ...	16 „ ...	Recovered...	Malarial Fever.
M	Seaman	„ ...	13 „ ...	„ ...	„

AUGUST—*continued.*

Sex.	Occupation.	Nationality.	Period as In-Patient.	Result.	Disease.
M	Steward...	English ...	15 days...	Recovered...	Malarial Fever.
M	Seaman...	„ ...	12 „ ...	„ ...	„
M	Steward...	„ ...	74 „ ...	„ ...	Malaria & Pleurisy.
M	Seaman...	Italian ...	26 „ ...	„ ...	Malarial Fever.
M	„	„ ...	26 „ ...	„ ...	„
M	„	Norwegian...	21 „ ...	„ ...	„
M	„	„ ...	3 „ ...	Death ...	„
M	Steward...	English ...	28 „ ...	Recovered...	„
		SEPTEMBER			
M	Fireman	English ...	7 days...	Recovered...	Malarial Fever.
M	„	„ ...	12 „ ...	„ ...	„
M	Baker	„ ...	35 „ ...	„ ...	„
M	Steward...	„ ...	11 „ ...	„ ...	„
M	Butcher	„ ...	22 „ ...	„ ...	„
M	Fireman	„ ...	27 „ ...	„ ...	„
M	Seaman...	Norwegian...	19 „ ...	„ ...	„
M	Steward...	English ...	15 „ ...	„ ...	„
M	„	„ ...	23 „ ...	„ ...	„
M	—	„ ...	8 „ ...	„ ...	„
M	Fireman	„ ...	9 „ ...	„ ...	„
M	Seaman...	„ ...	8 „ ...	„ ...	„
M	Soldier	„ ...	42 „ ...	„ ...	Hepatic Abscess.
M	Seaman...	„ ...	15 „ ...	„ ...	Malarial Fever.
M	„	„ ...	17 „ ...	„ ...	„
M	Cook	„ ...	8 „ ...	Relieved ...	Malarial Cachexia.
M	Fireman	„ ...	6 „ ...	„ ...	„
		OCTOBER			
M	Seaman...	English ...	9 days...	Relieved ...	Scurvy.
M	„	„ ...	29 „ ...	„ ...	Malarial Fever.
M	„	„ ...	13 „ ...	Recovered...	„
M	Steward...	„ ...	14 „ ...	„ ...	„
M	Fireman	„ ...	18 „ ...	„ ...	„
M	Seaman...	„ ...	26 „ ...	„ ...	„
M	Steward...	„ ...	25 „ ...	„ ...	„
M	Seaman...	„ ...	19 „ ...	„ ...	„
M	Fireman	Dutch ...	14 „ ...	„ ...	„
M	Seaman...	English ...	53 „ ...	„ ...	„
M	Fireman	„ ...	20 „ ...	„ ...	„
M	„	„ ...	6 „ ...	„ ...	„
M	„	„ ...	5 „ ...	„ ...	„
M	Seaman...	„ ...	8 „ ...	„ ...	„
M	Cook	Lascar ...	15 „ ...	„ ...	Diarrhœa (Trico-cephalus Dispar).
M	Trimmer	English ...	8 „ ...	„ ..	Malarial Fever.
M	Ship's Mate	„ ...	8 „ ...	„	„
M	Trimmer	„ ...	15 „ ...	„ ...	„
M	Ship's Officer... ...	„ ...	25 „ ...	„ ...	„

NOVEMBER

Sex.	Occupation.	Nationality.	Period as In-Patient.	Result.	Disease.
M	Carpenter	Norwegian	21 days	Recovered	Malarial Fever.
M	Seaman	Spanish	22 „	„	„
M	Fireman	English	16 „	„	„
M	„	„	6 „	„	„
M	Steward	„	17 „	„	„
M	„	„	18 „	„	„
Female	Stewardess	„	22 „	„	Dysentery.
M	Carpenter	„	8 „	„	Malarial Fever.
M	Trimmer	„	8 „	„	„
M	Trader	„	14 „	„	„
M	—	„	29 „	„	„
M	Mate	„	26 „	„	„
M	Fireman	Chinese	2 „	Death	Beri-beri.

DECEMBER

Sex.	Occupation.	Nationality.	Period as In-Patient.	Result.	Disease.
M	Seaman	English	6 days	Recovered	Malarial Fever.
M	Labourer	„	24 „	Relieved	„
M	Fireman	„	2 „	Recovered	„
M	Seaman	„	2 „	Relieved	„
M	Ship's Officer	„	9 „	Recovered	„
M	Naval Officer	„	—	—	„
M	Fireman	„	—	—	„
M	Dispenser	„	—	—	„
M	Ship Surgeon	„	—	—	„
M	Artillery Lieutenant	„	—	—	Beri-beri.
M	Seaman	„	—	—	Malarial Fever.
M	Steward	„	—	—	„
M	Seaman	Finn	—	—	„

DEATHS

1 Case	Sprue.
1 „	Dysentery.
1 „	Beri-beri.
2 „	Malarial Fever.
1 „	Blackwater Fever.

NATIONALITIES

English	151
Norwegian	5
German	3
Finnish	3
Swedish	2
Lascar	3
Italian	2
American	1
Dutch	1
Spanish	1
Malay	1
Austrian	1
Chinese	2
	176

LIBRARY

PERIODICALS TAKEN BY DEPARTMENTAL LIBRARY

Arbeiten aus dem Kaiserl. Gesundheitsamte.
Archives Orientales.
Archiv f. Schiff u. Tropen-Hygiene.
Archiv f. Hygiene.
Archives of Neurology.
Archives de Parasitologie.
Archiv f. Thierheilkunde.
Beiträge z. Pathol. Anat.
Brain.
British Medical Journal.
Centralbl. f. Bakteriologie (Pts. I and II).
Centralbl. f. Pathol. Anatomie.
Deutsche Medicinische Wochenschr.
Hygienische Rundschau.
Journal of the Agricultural Society.
Journal of Pathology.
Journal of Physiology.
Neurologisches Centralblatt.
Public Health.
Revista Medica de S. Paulo.
Sanitary Inspector.
Veroeffent. des Kaiserl. Gesundheitamts.
Vierteljahrschr. f. Gerichtl. Medicin.
Zeitschr. f. Fleisch u. Milch Hygiene.
Zeitschr. f. Hygiene.

BOOKS, ETC., ACQUIRED OR PRESENTED DURING 1899

*Boerhaave Laboratoire, 'Travaux,' 2 vols.
Duclaux, 'Traite de Microbiologie,' vol. ii.
*Government Reports:
Colonial Office, 'State Board of Health.'
India Office, 'Report of Sanitary Commissioner.'
'India Plague Commission,' chap. iv.
Admiralty, 'Report of the Health of the Navy.'
Board of Agriculture, U.S.A., 'Reports of Bureau of Animal Industry.'

* Presented.

Kirchner, 'Mikroskopische Pflanzen des Süsswassers.'
König, 'Verunreinigung der Gewässer,' 2 vols.
Lafar, 'Technical Mycology.'
Mez, 'Mikroskopische Wasseranalyse.'
*Municipal Reports :—
Berlin, 'Sewage Farm.'
Hamburg, 'Municipal Laboratory.'
Leipzig, 'Annual Report.'
Liverpool, 'Report of Medical Officer of Health.'
Manchester, 'Sewage Experts' Report.'
Minnesota, 'State Board of Health.'
Vienna, 'Report of Board of Health.'
Wiesbaden, 'City Engineer's Report' (3 years).
Newsholme, 'Public Health and its Applications.'
*New York State Hospitals, 'Pathol. Institute Contrib.,' 2 vols.
Paris, 'Annuaire de l'Observatoire Municipale.'
Pearmain & Moore, 'Applied Bacteriology.'
Pettenkofer's 'Festschrift.'
Pistor, 'Gesundheitswesen,' 2 vols.
Santo Crimp, 'Sewage Disposal.'
*Trans. 'Pan-American Medical Congress,' 1893, 3 vols.
*Upsala 'Universitats Arsskrift,' 5 papers.
Zopf, 'Die Pilze.'

BOOKS PURCHASED BY THE SCHOOL OF TROPICAL MEDICINE, 1899

Abel, 'Taschenbuch für den Bakteriologen.'
Anderson, 'Yellow Fever in West Indies.'
Blanchard, 'Zoologie Medical.'
Brault, 'Hygiene des maladies dans les Pays Chauds.'
Carter (Vandyke), 'Spirillum Fever.'
Celli, 'La Malaria.' (Annali d'igene sperimentale.)
Celli, 'Studio dell' Epidemiologia della Malaria.'
Celli, 'Sull' immunita dell' Infezion Malaria.'
Cross, 'Malarial Fever.'
Corres, 'Maladies des Pays Chauds.'
Davidson, 'Geographical Pathology.'
Dawson, 'Hygiene and Diseases of Warm Climates.'
Duncan, 'Prevention of Diseases in Tropical Campaigns.'

* Presented.

Fayrer, ' Tropical Diseases.'
Friedburger u-Frohner, ' Pathologie der Hausthiere.'
Grawitz, ' Pathologie des Blutes.'
Grawitz, ' Methodik der Blutuntersuchung.'
Grimm, ' Beriberi.'
Grunbert, ' Les Serums Therapeutiques.'
Haffkine, ' Report on Plague.'
Hansen and Looft, ' Leprosy.'
Hehir, ' Observations on the Hæmatozoon of Malaria.'
Hughes, ' Malarial Fever.'
Hulnebosch, ' Diagnostik der menschlichen Fæces.'
Impey, ' Leprosy.'
Kelsch and Kiener, ' Maladies des Pays Chauds.'
Koch, ' Reise-Berichte.'
Kilt, ' Bakterienkunde.'
Kartulis, ' Dysenterie.'
Laveran, ' Paludisme.'
Laveran, ' Traité de Paludisme.'
Leuckart, ' Parasites of Man.'
v. Limbeck, ' Klin. Pathologie des Blutes.'
Lee, ' Microtomists Vade Mecum.'
Legrain, ' Fièvres des Pays Chauds.'
Legrain, ' Notes sur la Pathologie speciale des indigines Algeriens.'
Lister, ' Mycetozoa.'
Mannaberg, ' Die Malaria Krankheit.'
Mannaberg, ' Die Malaria-Parasiten.'
Manson, ' Filaria Sang. Hominis.'
Manson, ' Tropical Diseases.'
Packard, ' Text Book of Entomology.'
Parke, ' Guide to Health in Africa.'
Pellegrin, ' Les poissons veneneux.'
Pfeiffer, ' Protozoen als Krankheitserreger.'
Pfeiffer, ' Protozoen Forschung.'
Pisani, ' Relapsing Fever.'
Plehn, ' Die Kamerun Küste.'
Plehn, ' Malaria Studien.'
Plehn, ' Malaria in Kamerun.'
Rogers, ' Report on Kala-azar.'
Roux, ' Les Maladies des Pays Chauds,' 3 vols.

OYSTERS AND DISEASE

LANCASHIRE SEA-FISHERIES MEMOIR—No. I.

OYSTERS AND DISEASE

AN ACCOUNT OF CERTAIN OBSERVATIONS UPON THE NORMAL AND PATHOLOGICAL HISTOLOGY AND BACTERIOLOGY OF THE OYSTER AND OTHER SHELLFISH.

BY

W. A. HERDMAN, D.Sc., F.R.S.,

PROFESSOR OF ZOOLOGY IN UNIVERSITY COLLEGE, LIVERPOOL, AND HONORARY DIRECTOR OF SCIENTIFIC WORK TO THE LANCASHIRE SEA-FISHERIES COMMITTEE;

AND

RUBERT BOYCE, M.B.,

PROFESSOR OF PATHOLOGY IN UNIVERSITY COLLEGE, LIVERPOOL, AND BACTERIOLOGIST TO THE CITY OF LIVERPOOL.

With Eight Plates.

LONDON:
GEORGE PHILIP & SON, 32 FLEET STREET, E.C.
LIVERPOOL: PHILIP, SON & NEPHEW, 45—51 SOUTH CASTLE STREET.
1899.

Dedication.

To

JOHN FELL, Esq., J.P. and D.L.,

Chairman of the Lancashire Sea-Fisheries Committee.

Dear Mr. Fell,

We think it most appropriate, and it is a pleasure to us, that this First Lancashire Sea-Fisheries Memoir should be dedicated to you, the First Chairman of the Committee under whose auspices a large proportion of this and other scientific work on Fisheries' questions has been carried on.

It is widely known in Lancashire that the formation of our Local Sea-Fisheries Committee was largely due to your personal energy and influence, and that to your wise guidance during the first decade of its existence is due in great measure the success of its administration, and its present commanding position. But it may be well to point out, what is not so generally known, that from the first you recognised that the Sea-Fisheries form a department of general Marine Biology, and that sound legislation and administration must be founded on the results of scientific and other correct practical investigations.

W. A. Herdman.
R. Boyce.

University College, Liverpool,
July, 1899.

CONTENTS.

		Page
I.	INTRODUCTION	1
II.	HISTORICAL SUMMARY	3
III.	MATERIAL AND METHODS	6
IV.	STRUCTURE OF THE BRANCHIÆ	7
V.	THE PROTRACTOR PEDIS MUSCLE	10
VI.	THE LIVER	13
VII.	GREEN OYSTERS	15
VIII.	IRON AND COPPER IN OYSTERS	21
IX.	MICRO-CHEMISTRY OF THE GREEN OYSTER	30
X.	EXPERIMENTS IN FEEDING WITH METALLIC SALTS	32
XI.	ON THE CONNECTION BETWEEN OYSTERS AND INFECTION	35
XII.	CONDITION OF OYSTER BEDS AND LAYINGS	39
XIII.	BACTERIOLOGY OF THE OYSTER AND OTHER SHELL-FISH	41
XIV.	CONCLUSIONS	53
	EXPLANATION OF THE PLATES	56

OYSTERS AND DISEASE.

I.—INTRODUCTION.

This research was commenced three years ago, and has been carried on intermittently in the intervals of other work. One of us (W. A. H.) had been working for some years previously at Fishery questions for the Lancashire Sea-Fisheries Committee, and so had become cognisant of the methods of growing and bedding Oysters on our coasts, of their variations in condition and colour, and of the want of exact knowledge as to their connection with disease. As this was clearly a matter of combined Natural History and Bacteriology, he proposed to his colleague (R. B.) that they should undertake a joint investigation of the structure and life-conditions of the Oyster in healthy and unhealthy environments—a matter of importance both for the Oyster industries and for public health.

A preliminary paper on the plan of work and on the first experiments was laid before Section D. (Zoology and Physiology) at the Ipswich meeting of the British Association (September, 1895). This led to the formation of a small Committee to carry on the work, with the help of a grant of £40; and interim reports were submitted at the Liverpool (1896) and Toronto (1897) meetings. The final report of that Committee was given to the British Association at Bristol last autumn.

In these reports, necessarily brief and without illustrations, it has only been possible to give a summary of results; and, in the summer of 1897, one section of the subject—the presence of copper in the leucocytes of certain Oysters—was treated by us in a preliminary manner in a short communication to the Royal Society.* We propose now to give a full account of the evidence upon which our various conclusions, some of which were announced in these preliminary reports, are based, along with a discussion of our results and those of other workers, both from the purely scientific and also from the Fisheries and the Sanitary points of view.

One member of our British Association Committee, Dr. Charles Kohn, Lecturer in Chemistry at University College, Liverpool, has given us considerable help from time to time in discussing with us the chemical aspects of the work, and in making analyses of different kinds of Oysters. We have also had the advantage of advice from our

* Proc. R. S., vol. LXII., No. 379, p. 30.

colleague Professor Sherrington, also a member of the Committee. Our friend Dr. P. P. C. Hoek, Scientific Fisheries Adviser to the Dutch Government, visited our laboratories in February, 1897, for the purpose of seeing our work and its bearings upon Dutch Oyster culture. He went through all our results with us, and took part in an examination at that time of various kinds of green oysters. We have recently (June, 1898) had the opportunity of discussing fully the histo-chemical reactions with Professor Macallum, of Toronto, whose opinion on some points will be referred to further on.

We desire to acknowledge, finally, the help we have received, in what has been a very laborious investigation, because of the large numbers of animals dealt with and the very different lines of work involved, from the Assistants in our laboratories. We would thank especially Mr. Andrew Scott and Mr. James Johnstone, "Fisheries" Assistants in the Zoological Laboratory; and Dr. Dutton, Dr. Christophers, and Dr. Abram, in the Pathological Department. Some of the figures in our plates have been drawn, under our direction, by Mr. Johnstone, and others by Mr. Fisher of the Pathological Department. The rest are our own.

LIVERPOOL,
December, 1898.

II.—HISTORICAL SUMMARY.

A considerable part of our work has dealt with "greenness" in oysters of different kinds—a subject on which many contradictory statements have been made, and which has a curiously involved history.

In 1866, Mr. A. W. E. O'Shaughnessy* gave an excellent account of our knowledge of the "greening" of oysters up to that date. He showed how M. Benjamin Gaillon, in 1820,† observed the blue diatom *Navicula fusiformis*, variety *ostrearia*—which he then called *Vibrio ostrearius*—in the "Claires" of the Oyster Merchants at Marennes, &c., in France, and attributed to it the dark bluish green tint of the gills, palps, intestine, and liver of the cultivated oyster. He also discussed the observations of Valenciennes, Dumas, Bizio, Buckland, and others; and cited some historical cases of poisoning supposed to be due to the presence of copper in large quantities in green oysters. As these earlier observations are discussed also by Professor Ray Lankester in his important paper in 1885,‡ they need not be further alluded to here. But we must add to Lankester's record by referring to Puységur,§ who, in 1880, gave an account of certain experiments, in which he stated that he had succeeded in turning oysters green in some few hours by feeding them upon the diatom *Navicula ostrearia*. It has also been shown experimentally by Bornet, Decaisne, and others, that white oysters can be greened rapidly by keeping them in clean soup plates and feeding them with water containing the *Navicula*. These interesting experiments were carried out at Le Croisic, in Brittany, and were afterwards repeated in Paris, and the result seems entirely opposed to the old suggestion, due originally to Coste,‖ in 1861, that iron salts in the soil at the bottom of the "Claire" are the cause of the greening, which suggestion was alluded to again more than twenty years ago by Bouchon-Brandely,¶ and has quite recently been revived by Carazzi** at Spezia. Moreover, Bornet and Ad. Chatin have shown that in the parks at Sable d'Olonne the greening may be suddenly manifested where the oysters had previously remained white, under circumstances where the microscopic fauna and flora of the water may well have changed, but where the floor of the park has undoubtedly remained the same. Finally, Mr. E. Newman, manager of the Colne River Oyster Beds, has shown us that when the Algæ (common Cyanophyceæ) in his ponds get into a certain condition, he can convert the ordinary colourless Colchester "native" into a green-gilled oyster in 24 hours.

---

* Ann. and Mag. Nat. Hist., vol. XVIII., p. 221

† Journ. de Physique, tome XCI., p. 222.

‡ Quart. Journ. Microsc. Sci., vol. XXVI., p. 71.

§ Revue Maritime et Coloniale, 1880.

‖ Voyage d'Exploration sur le littoral de la France, &c.

¶ Rapport au Ministre de la Marine relatif à l'Ostréiculture, &c., Paris, 1876.

** Mitth. Zool. Stat. Neapel. 1896, p. 381.

Ryder in America was also, in 1880, investigating the greening of oysters, with much the same results as those of Puységur. He went a step further, however, and showed that the colouring matter was taken up by the amœboid blood-cells, and that these wandering cells containing the pigment were to be found in the heart, in some of the blood vessels, and in aggregations in "cysts" under the surface epithelium of the body.* He describes the colour (in the ventricle) as a "delicate pea-green," and states that it is not chlorophyll or diatomine: he suggests that it may be phycocyanine or some allied substance. This is undoubtedly the same green in the leucocytes and the heart which we now find to contain a salt of copper.

In 1885, Ray Lankester† gave a useful summary of some of the earlier papers, and discussed the main questions concerned. Moreover, he investigated the gills of the green oyster histologically, and described and figured for the first time cells laden with green granules which occur in the epithelium of the gills and labial palps. He showed that such cells are also present in the common oyster, where, however, they are not green, and that these or similar cells may be found also wandering over the surface of the gills. He considered these as "secretion" cells, but those on the surface are clearly the same structures which Ryder a few years before had found in the blood, and are therefore leucocytes. Lankester found the *Navicula* in the intestine of the green oyster; and he reasserted that there was no copper and no iron in the diatom's refractory blue pigment—which he described under the name "Marennin," and identified with the pigment of the large green "secretion cells" of gills and labial palps.

This explanation of the "greening" has since been confirmed by Joannes Chatin,‡ who has also described more fully the histology of the large green granular cells of the gills, which he called "macroblasts." He showed that in brown oysters these same cells contain brown granules.

Lankester's amœboid cells, laden with green granules and moving over the surface of the gill, are almost certainly leucocytes or amœbocytes which have wandered out from the body through the surface epithelium: such diapedeses are probably of frequent occurrence in the Mollusca. Pelseneer§ (1892) and De Bruyne‖ (1895), in recent papers, consider that these cells act as phagocytes—which is quite probable—and we understand that Lankester¶ would now accept this view himself. Dr. D. Carazzi of Spezia, however, who has recently (1896 and 1897) written several rather controversial papers on the subject, will have none of this, and states that the diapedesis is entirely a pathological phenomenon. He attributes the green colour to the cylindrical epithelium solely, and not to the "secretion cells," or "macroblasts," and regards the Marennin as a nutrient

* Ryder's papers are found scattered through the U.S. Fish Commission publications from 1882 onwards.

† Quart. Journ. Micros. Sci., vol. XXVI., p. 71.

‡ Comptes-rendus, CXVI., p. 264 (1893); and CXX., p. 884 (1895).

§ Bull. Soc. Malacol. Belg., t. XXVII., p. lxii. ‖ Arch. de Biologie, t. XIV., p. 161.

¶ See *Nature* for 9th May, 1895, p. 28. Joannes Chatin, in a more recent note (Comptes-rendus, Feb. 24, 1896) adopts a modified view that the phagocytes are connective-tissue corpuscles distinct both from the blood-cells (leucocytes) and the fixed macroblasts.

material on its way inwards from the surface to the liver. However, as Carazzi contradicts* almost everything both morphological and physiological that has been stated by previous workers on the oyster—Lankester, J. Chatin, Pelseneer, De Bruyne, Ryder, and ourselves alike—it is a little difficult to meet him seriously and to find patience to deal in detail with all his remarkable statements. Some of the points he raises, however, and of his so-called errors in the work of others, will be discussed further on in this paper.

There are many other papers upon both the structure and the physiology of the oyster—and especially the green Marennes oyster—which we have not referred to above. A very complete bibliography of the subject was published by the United States Fish Commission in 1892, and so we have only considered it necessary to discuss the papers which we have found useful, or which for any reason seemed to require our attention. Previous work on the Bacteriology of Oysters, and the supposed connection with disease in Man, we shall discuss in the latter part of this paper.

* Mitth. Zool. Stat. Neapel. Bd. XII.; Internat. Monatsschrift fur Anat. u. Physiol. Bd. XIV., &c.

III.—MATERIAL AND METHODS.

We have used three distinct species of oyster, and many local varieties, or races, from various parts of the European and American coasts. The species are the following :—

1. *Ostrea edulis*, Linn., the common edible oyster of North-west Europe, the species to which our "Natives," "Deep-sea oysters," "Rock oysters," "Pandores," and many other kinds from our own and neighbouring coasts belong. We have had samples of these from Colchester, Pyefleet, Mersea, Roach River, Leigh, Plymouth, Falmouth, Jersey, Wales, Isle of Man, Lancashire, the Scheldt, Nieuport, Spezia, and the West Coast of France.
2. *Ostrea virginica*, Gm., the American oyster: very commonly "laid," "bedded," or stored on various parts of our coast. We have had samples of "East Rivers," "Sounds," and "Blue Points," direct from America to Liverpool; and of those that have been bedded in this country for longer or shorter periods, from Dublin, Carlingford, Fleetwood, Brightlingsea, Leigh, Falmouth, and the Menai Straits.
3. *Ostrea (Gryphæa) angulata*, Lamk., the Portuguese oyster of Southern and Western Europe. We have had samples of this from Arcachon in the South-west of France, and from Falmouth, Leigh, and the estuary of the Colne.*

All the oysters have been examined in the first place alive, and their characters noted: coloured sketches have been made of many. In some cases the gills and other parts have been examined fresh, in teased and pressure preparations; but in all cases pieces have also been fixed, preserved, and sectioned. We have employed a variety of methods. Some gills and pieces of liver and intestine have been frozen in gum and cut at once with the freezing microtome, in order to have sections of the fresh tissue unstained for comparison with the sections which have undergone treatment with re-agents.

We have obtained the best results from our sections of mantle lobes, gills and viscera which had been fixed in corrosive sublimate and acetic acid, washed with alcohol, and then put through increasing strengths of alcohol, and finally iodine dissolved in 90 per cent. alcohol, cleared in either turpentine or xylol, imbedded in paraffine, cut with the Cambridge Rocking microtome, stained in eosine and hæmatoxylon, and mounted in balsam. Some of our preparations were fixed in 5 per cent. solution of formol in place of corrosive and acetic. The washing with iodine and alcohol was sometimes deferred until the sections were on the slide, previous to staining.

The Histo-Chemical and Bacteriological methods will be stated below in Sections IX. and XIV. respectively.

---

* We are much indebted to several gentlemen for kindly taking trouble to supply us with the special kinds of Oysters we desired. We would especially thank Charles Petrie, Esq., C.C., and Geo. T. G. Musson, Esq., of Liverpool; Rupert Vallentin, Esq., of Falmouth; Dr. P. P. C. Hoek, of Helder; Dr. J. G. de Man, of Ierseke, and Dr. J. M. Bottemanne, of Bergen-op-Zoom, in Holland; and also Dr. Murie, and Mr. James Hornell of Jersey.

IV.—THE STRUCTURE OF THE BRANCHIÆ.

(See Plate I.)

Figures 2 to 5 on Plate I. show the normal structure of the gill in a healthy oyster, whether white or green. The oyster has the four great flattened branchial folds (Pl. I., Fig. 1, *br.*), an external and an internal on each side, characteristic of Lamellibranch Molluscs. On account, however, of the absence of the foot, the two internal branchiæ come into close proximity in the middle line along their whole length, and so all four gills lie closely superposed along the ventral and posterior edges of the body: they encircle about two-thirds of the circumference. The branchiæ occupy about one-third of the superficial area exposed on removal of a shell, and make up from one-third to one-sixth of the total bulk of the body. Physiologically they are one of the most important and constantly active organs of the body, as respiration is certainly not their sole, probably not their chief, function. They cause, by their ciliated epithelium, that powerful and all important current of water which carries food as well as oxygen into the body and removes all waste products.

The gill filaments constituting the gills hang side by side; but even those of one gill lamella, or surface, are not all in the same vertical plane, as the row of filaments is plicated to form alternate crests and troughs (Pl. I., Fig. 2). The crests or projections form ridges visible to the eye, of which there are about one hundred or rather more on the surface of a gill; and as each such crest has at least 10 (sometimes 15) gill filaments, there must be over 1,000 filaments on each surface of each gill, or, say, 8,000 to 10,000 in all. The transverse sections shown in Figures 2 and 3 show clearly the arrangement of the filaments in projecting crests on both surfaces of each gill. Concrescence (or inter-filamentar junctions) takes place to various degrees between neighbouring filaments, and consequently the blood channels form a network of varying arrangement in different parts, and the water passages are in many places partially occluded. In Figure 3, *a* indicates a spot where the filaments retain their primitive independence; while *b* shows complete concrescence between the 12 filaments of one crest. This Figure and the other transverse sections show also the arrangement of the skeletal elements in the connective tissue of the filaments. Each filament has two longitudinally running skeletal bars, formed of condensed fibrillated connective tissue, roughly fusiform in section, and lying just under the epithelium on the external face of the filament (Figs. 4 and 5). At the bottom of each trough we find a single enlarged filament, with a modified pair of skeletal bars of much greater size, and of triangular form in cross-section. These pairs of larger bars in each trough are conspicuous objects in low-power views (*see* Figs. 2, 3).

Each gill filament has the branchial epithelium (ectoderm) on its outer surface, and the blood channel, lined by a thin layer of connective tissue, in its interior (Fig. 4). The skeletal bars lie between the epithelium and the connective tissue; the blood space contains here and there leucocytes, rounded or stellate, for the most part colourless, blood cells. There are then the following four tissues :—

1. The branchial epithelium.
2. The skeletal bars.
3. The connective tissue.
4. The blood cells; and also certain eosinophilous cells.

The Epithelium.—This varies in appearance in different parts, and requires further description. It has been figured and described most satisfactorily in the past by Janssens* and by De Bruyne†, and we also show it in Figs. 4, 5, and 10, on Pl. I. A tract of the epithelium on each side of each filament bears especially long cilia, and these tracts are separated by a narrow non-ciliated zone from the front or free edge of the filament, which is covered by a uniform band of short cilia (Fig. 5). The depression between adjacent filaments, where they join, is never ciliated. The nuclei of all these epithelial cells are large and ellipsoidal (Fig. 10). The cells on the prominent free edge of the filament are of tall, columnar form; at the base and on the interfilamentar junctions they are cubical, squamous epithelium. Amongst these epithelial cells we find the large rounded granular cells, which were described by Lankester as secretion cells, by J. Chatin as "macroblasts," and by others as "Beckerzellen." They are clear and refracting in the white oyster, but distinctly green-coloured in the green oyster. They give a marked eosinophilous reaction (Fig. 5), and will be referred to again below in that connection.

The Skeletal Bars lie two in each gill filament, inside the epithelium and near the centre, in a section (*see* Fig. 5, etc.). They diverge outwards towards the outer edge of the filament, and either join or come into close proximity in the middle line of the free edge. They occasionally come in contact at their inner ends also (*see* Fig. 5). In the trough between two of the series of projections formed of filaments, the larger modified gill filament (the "segment primaire" of Janssens) has a pair of especially thickened skeletal bars of wedge-like shape, in section, and set at a wide angle to one another (Figs. 2 and 3). It is only these larger gill filaments, and usually only the alternate ones of these, that are continued into inter-lamellar junctions (Fig. 3).

The Connective Tissue forms a thin layer, in which the skeletal bars lie, inside the epithelium, and bounding the blood-channels in the branchial filaments and their junctions. It is a membrane nearly homogeneous or slightly fibrillated, with occasional fusiform or stellate connective-tissue cells, the nuclei of which are seen stained in the

* Les Branchies des Acephales. La Cellule, t. IX. fasc. 1, p. 19 (1893). *See* also Kellogg, U.S. Fish Commission Bulletin, vol. X. (1892).

† Arch. de Biologie, t. XIV. (1895).

sections. Here and there is found a large granular wandering cell, markedly eosinophilous, and precisely similar in character to those seen in the connective tissue of the mantle and those scattered through the epidermis of both mantle and branchiæ. These are to be regarded as granular leucocytes on their way to the exterior. They are shown in Figs. 9 and 13.

The Blood Cells are normally colourless leucocytes, measuring on the average 10 μ in diameter (Fig. 11). Some, however, are granular, and the granules may be yellow, brown, black, or green in colour. In diseased oysters, showing the green leucocytosis described below, the number of opaque granular leucocytes seems to become largely increased, and they are of a pale chalky-green colour. With osmic acid a black reaction is occasionally given by the granular leucocytes. In blood film preparations we have repeatedly tried to obtain evidence of an eosinophilous reaction in the normal leucocytes, but without success. On the other hand, the larger wandering cells in the tissues, or amœbocytes, are markedly eosinophilous.

Eosinophilous Cells.—Although we have not observed eosinophilous leucocytes in the blood-vessels, there are numerous cells in the tissues which give an eosinophilous reaction. Large ovoid cells are met with, scattered with some regularity along the free edge of the gill filaments (Pl. V., Fig. 3), and these, from the frequency of their occurrence, seem to be fixed elements of the epithelium ("Beckerzellen"). Corresponding cells in the mantle (Pl. V., Fig. 2) and intestinal epithelium have usually a pyriform appearance, and sometimes appear to be discharging their granular contents on the free surface. In the green American oysters described below, we have sometimes observed that these cells give a well-marked reaction with pure dilute hæmatoxylin (Pl. V., Fig. 8). There are also certain eosinophilous wandering cells which are found beneath the epithelium of the intestine, mantle, and branchiæ, and which, in some cases, are seen making their way through the epithelium to the exterior (Pl. V., Figs. 4 and 5); while other wandering cells—the green leucocytes of the American oyster—are never, in our experience, eosinophilous. The eosinophilous reaction, in fact, indicates merely a condition in which various epithelial and wandering cells may be found.

V.—THE PROTRACTOR PEDIS MUSCLE.

(See Plate II.)

In opening large numbers of American oysters, usually a hundred at a time, we noticed the constant presence of a more or less distinct spot (Pl. II., Fig. 2, *sp.*) on the mantle, near the anterior end of the visceral mass, and of a slight depression or mark on the shell over the spot (Pl. II., Fig. 1, *sp.*). The spot is circular, and about 2 mm. in diameter; it is rather lighter and less opaque than the surrounding mantle, and its edges may be coloured with a deposit of dark brown or black pigment granules (*see* Pl. II., Fig. 3). At first we were inclined to think this the opening of a gland, or an aggregation of minute glands, as a watery fluid continued for some time after opening the oyster to ooze from the spot, and collect in a drop, which re-formed on removal. Dissection and the examination of sections showed, however, that we had to do with a band of muscular tissue, and that the "spot" was the insertion of the muscle, and the mark on the shell the scar produced by that insertion. This insertion mark has exactly the appearance and structure of the adductor impression. (Compare Figs. 4 and 5 with 6 and 7, on Pl. II.).

This muscle has been noticed before. J. A. Ryder,* in 1884 and 1893, writing of the mark on the shell, says: "It gives attachment to a feeble muscular bundle which springs out of the mantle on either side of the visceral mass, and when the animal is torn loose a slight whitish scar on the soft part marks its position on the surface of the mantle. I have been informed that Mr. W. H. Dall, who has investigated the matter, has identified this muscle with the pedal muscle of some other acephalous molluscs." R. T. Jackson, in his Phylogeny of Pelecypoda,† figures what is clearly our spot as a pedal muscle; but it is not mentioned by Kellogg in his work on the Morphology of Lamellibranchiate Mollusks.‡

Huxley showed, in 1883, that the first formed adductor muscle of the oyster, seen in the free-swimming larval stages, cannot from its position be the adductor present in the adult. The larval muscle, which is clearly an "anterior adductor" muscle, lies antero-dorsally in the body, and would require to traverse the alimentary canal to attain to the position of the adult muscle. Huxley predicted that a new second adductor muscle would be found to develop in the young oyster, and he argued, therefore, that oysters must be derived from dimyarian ancestors, and that their single large muscle is the persistent posterior adductor muscle, the anterior one being lost in early life. Jackson's discovery of a transitory dimyarian stage in the very young American oyster *(Ostrea virginica*, Gm.*)* proves the truth of Huxley's view.

---

* Contributions to the Life-History of the Oyster, 1893, p. 719.

† Memoirs Boston Soc. Nat. Hist., vol. IV., No. VIII., 1890.

‡ Bulletin U.S. Fish Commission, vol. X. (1892).

The idea that the small muscle passing inwards from the "spot"—which in position is a good deal anterior to the functional adductor—might be the reduced remains of the larval anterior adductor, is at once negatived by the observation that the fibres do not pass through the body from side to side, as adductor fibres must; but that we have to do with two distinct muscles, one on each side of the body. This, then, suggests the muscles for moving the "foot," and although there is no foot in the adult oyster, there can be no doubt that Ryder and Jackson were right in referring to this as a "pedal" muscle.

The muscles which may be present in other Lamellibranchs in relation to the foot are a protractor and an anterior and a posterior retractor on each side. The posterior retractor is out of the question. The protractor and the anterior retractor are usually inserted into the shell a little posterior to the anterior adductor muscle, and the protractor is the more ventrally placed of the two. The hinge line of the oyster is anterior, and if we may judge from Horst's figure of the larval oyster at the stage when the foot, as a ciliated ventral projection between the mouth and the anus, is in its most fully developed state, a protractor muscle would run posteriorly from in front of the mouth or œsophagus; while a retractor would, from what we know of it in other molluscs, probably be placed more dorsally, so as to cross the stomach. Our muscle runs antero-posteriorly, and is towards the ventral part of the visceral mass, close to the insertion of the labial palps. Such indications as we have, then, point to the "spot" being the place of insertion of the modified remains of the protractor pedis muscle.

The foot is however lost in the oyster at a very early period—while the larva is still free-swimming; and if the protractor muscle is present in the adult in this relatively large condition, it must be because it has become adapted to a new purpose. We have traced the muscle fibres in the American oyster by means of serial sections from the insertion into the shell (Pl. II., Figs. 9 and 10) at the "spot," posteriorly along the top or attached edge of the outer labial palp, to the anterior end of the branchial lamellæ. The fibres first run inwards, and then obliquely backwards (Fig. 11), and come into close relation with a large blood sinus (*see* Pl. II., Figs. 11, 12, 13, *lac*), which lies in the mantle just over the attachment of the outer labial palp. The muscle then works its way round the sinus, so as to appear upon its ventral, and finally its inner face (Fig. 12). It thus comes to lie internal to some of the renal tubules, and close to the outermost cæca of the liver. Our muscle now crosses at right angles a bundle of muscle fibres running from the mantle downwards ventrally into the palp, and containing a large nerve, as is usual with the muscle bundles of the mantle. Finally, having reached the level of the posterior end of the palp, our muscle turns ventrally, and begins to spread out (Fig. 13) in the sub-epithelial tract of mesodermal tissue which lies between the top of the mantle lobe and the attachment of the gill lamellæ, and which is morphologically a part of the stem of the ctenidium. Most of the fibres end in the connective tissue, immediately under the epidermis, between the descending (internal) and the ascending (external) lamellæ of the outer gill. The whole course of the muscle is about 6 mm. long in a moderately large oyster; it is about 1·5 mm. in diameter where inserted into the shell, and less than half that size when it reaches the branchiæ.

As to the probable function of this muscle in the existing oyster, its action clearly must be to draw the labial palps and the anterior end of the branchiæ forwards and outwards. When this action is imitated by means of forceps on the fresh oyster, the result is seen to be that the funnel leading to the mouth is widened and opened, and the "catchment area" by which the mouth is supplied becomes increased. We give a diagram, in Fig. 14, showing the relations of the protractor muscles of the two sides to the mouth and neighbouring parts, and we add in dotted lines the probable course of the muscles before the foot disappeared in ancestral oysters. It seems to us that when the muscle was no longer needed as a protractor pedis, it may readily have come to serve its present useful purpose by the posterior half of the muscle aborting, while the anterior half remained and acquired an attachment to the connective tissue of the palp and the gill through which it must have formerly passed, and where it is quite likely some of the fibres always ended.

Our figure (Pl. II., Fig. 14), which, although drawn as a diagram, is true in its proportions, and was sketched directly from the living animal, shows clearly the configuration of the labial palps when the two protractor muscles are slightly drawn upon. The inner or posterior palps curl together in the middle line behind the mouth, while the outer or anterior palps diverge widely. The anterior ends of both gills on each side are received between the outer and inner palps of that side; so that the only avenue to the mouth, the passage on each side between the two palps, becomes much widened at its posterior end, more funnel-shaped, and evidently better adapted for collecting the food particles from the anterior ends of the gills, and passing them forward to the mouth.

The action of the oyster in opening its shell (*i.e.*, divaricating the valves), preparatory to feeding, separates the points of attachment of the two muscles, and so of itself, even without any muscular contraction, causes the opening up of the food avenues described above: the contraction of the muscles which doubtless takes place will emphasize the same action.*

* The technical term "fishing" used by oyster-growers and others connected with the trade, to indicate the act of feeding in an oyster, is therefore so far a correct description, inasmuch as the action is comparable with the spreading of a net.

VI.—LIVER.

(See Plate III.)

The large gastric gland commonly called "liver" in Mollusca is of course well known to be neither homologous nor strictly analogous with the liver of vertebrates. As the sole digestive gland acting upon all constituents of the food, it is more nearly analogous with the pancreas of higher animals, and has sometimes been called a hepato-pancreatic gland. We are, however, familiar in some of the higher invertebrates with glandular cæca of the mesenteron which act as stomach diverticula, and apparently receive, digest, and absorb a portion of the food. This may well be the case to some extent with the liver of the oyster. It may possibly have an absorbing as well as a secreting function to perform in connection with digestion, and it is highly probable that, in addition, it has important metabolic functions in connection with the elaboration and storing up of reserve materials, including pigments, from the food.

The liver communicates with the stomach by means of a wide passage on each side (Pl. III., Figs. 4 and 5), which almost at once divides (Fig. 13) into several branches or hepatic ducts leading to the cæcal lobules, which are lined by the hepatic cells. The ducts, as was pointed out by Ryder in 1884, are lined by ciliated epithelium resembling that of the stomach (compare Figs. 14, 15, 16, &c.), and so can always be easily distinguished in sections from the cæca, the walls of which are formed by the large irregular hepatic cells (Figs. 6, 7, 8, &c.).

There is a considerable difference in the size of the liver and in the number of lobules or cæca in different oysters, and, moreover, the size of the cæca and of the hepatic cells seems to vary with the condition of nutrition of the animal, and also becomes reduced at the breeding time. One of the first points we noticed in connection with the green leucocytosis of certain American oysters, was, when examining the animals fresh, that they seemed to us to have the liver in a shrunken and degenerate condition. Carazzi objects to our use of this phrase, and seems to think that the liver is always in the same condition. Even a superficial examination of a large number of oysters, at different times and from different localities, is sufficient to show that he is mistaken, and that the "good conditioned" appearance of the oyster depends largely upon the state of the liver, as well as of the vesicular connective tissue around it. We now show by our figures the comparison between the healthy (Fig. 10) and the unhealthy (Fig. 11) liver, the latter showing the condition which we meant to indicate by the terms "shrunken and degenerate" in our preliminary report.

The colour also of the liver varies greatly, and may be dark brown (Figs. 1 and 2), bright yellow brown (Fig. 3), or dull green. The liver cells stain readily with Ehrlich's hæmatoxylin, and with osmic acid granules can be demonstrated in abundance

(Fig. 9). Barfurth and Frenzel have shown the presence of three kinds of cells in the glandular epithelium of the molluscan liver, viz.—"Körnerzellen," "Fermentzellen," and "Kalkzellen"; but not all of these are present in all cases. Frenzel* states in regard to the common oyster:—"(*a*) Die Körnerzellen haben einen Durchmesser von circa 32 mikr. Die Körner sind gelb- bis röthlich-braun gefärbt, 3 bis 4 mikr. gross und kugelig. Die granula sind wenig zahlreich und punktförmig. (*b*) Ferment- (Keulen-) Zellen nicht aufgefunden (März). (*c*) Kalkzellen fehlen."

We find two kinds of glandular cells in the liver of our oysters—the ordinary large granular liver cells (Körnerzellen), and smaller, more deeply-staining cells, which we take to be Frenzel's "Fermentzellen." The relative positions, sizes, and appearance of these cells is shown in Figs. 6, 7, 8, &c., on Pl. III. We have in some cases (Fig. 12) observed the presence of small green pigment† granules in the larger cells, but have not found any giving an iron reaction. Here and there, in the vesicular connective tissue between the cæca, may be seen (Fig. 10, *e. chl.*) the aggregations of dark green granules described as "entero-chlorophyll" by MacMunn. Whether this pigment is a modification of the yellow hepatochrome described by Miss Newbigin in the liver and integument of Crustacea, and which apparently may vary from red to green in colour, or is a derivative of chlorophyll from vegetable food, as held by MacMunn, and this seems very probable, cannot be determined without further investigation both spectroscopic and chemical. In the meantime, enterochrome (*see* Newbigin, Quart. Journ. Mic. Sci., November, 1898) certainly seems the more appropriate name for this pigment, or set of pigments.

* Mikrographie der Mitteldarmdrüse (Leber) der Mollusken. Nova Acta Leop.-Carol. Akad., Bd. XLVIII., No. 2, 1886.

† Ryder also described green pigment in the inner ends of the liver cells in 1893.

VII.—GREEN OYSTERS.

(See Plate IV.)

The discovery of a green tinge of more or less intensity, which made its appearance on the mantle and other parts of the body of some of the oysters in our experiments, started us on a series of investigations into the minute structure of the green parts, and the nature and causes of greenness in general in oysters. One of the first conclusions we arrived at was that there are several distinct kinds of greenness in oysters, the confusion between which in the past has led to differences of opinion and unfortunate controversies. The colouration has been attributed to the most varied causes by different investigators. It has been localised in mantle, gills, palps, heart, liver, intestine, or any two, three, or all of these parts. It has been stated at various times to be due to copper, iron, chlorophyll, entero-chlorophyll, "bile-pigment," phyco-cyanine, and to the pigment of certain diatoms, and all these statements have again been contradicted. Probably each of them was more or less correct of the particular oysters or organs examined by their authors. It is unfortunate that some writers have given rise to considerable confusion and misapprehension by not confining their conclusions to the kind of oysters they investigated.

The green of our experimental oysters we saw at once was a very different matter from the rich blue-green of the healthy Marennes oyster; certain green Falmouth oysters are probably different from both; the Roach River green oysters are merely a paler variety of the Marennes greenness; and, again, there is a sickly grey-green tint seen in some Dutch oysters when they get into poor condition which is not exactly the same as any of the above.

Most of our work has been done on the pale green American oysters relaid on the Lancashire Coast; but we have also taken occasion to examine into the other varieties of greenness seen in the Marennes, the Roach River, the Falmouth, and the Dutch oysters. We have had Marennes and Dutch oysters sent to us direct from France and Holland, and one of us (W. A. H.) has visited all the chief oyster-culture stations on the French and Dutch Coasts.

1. **Green Oysters of Marennes** (Pl. IV., Fig. 1).—We agree with practically all previous investigators in regarding these oysters as being in a healthy condition—although green. Whether the green substance is derived from food taken in by the mouth, or from nutriment absorbed by the surface epithelium, there can be little doubt that it is a product of metabolism stored up in certain large granular cells met with in liver, intestine, and gills. It has been said to contain a small amount of iron, and is entirely different in its reactions from the copper-containing green pigment we have met with in some other oysters. With certain modifications, we accept Lankester's

account of the greenness of the Marennes oysters as being correct. Carazzi, in his recent paper, makes a great point of the contents of Lankester's "secretion cells" ("Beckerzellen") of the branchiæ being always colourless, and he speaks of the error into which Lankester, Chatin, and others have fallen in considering these large globular cells to be sometimes loaded up with green granules. In this Carazzi is undoubtedly wrong. Although in ordinary colourless oysters the "secretion cells" are clear and colourless, in most green-gilled oysters (those of the Roach River in England, and some Dutch, as well as the Marennes) we find them to be distinctly green (Pl. IV., Figs. 3, 4); and we are able to support Lankester in his views as to the origin of the colour, which Carazzi has attempted to demolish.

Carazzi says that an occasional green appearance in the secretion cells may be due to an optical illusion, the green of the neighbouring epithelium appearing by refraction to be in the large clear cell. We only mention this in order to state that we have provided against any such optical illusion. We have seen the green colour of the secretion cells quite distinctly, not only in surface views and in sections, but also in the isolated cells, in teased preparations of the living tissue. We have been able to show them in this condition to several of our colleagues, and all have agreed that the green colour, although of course faint in single cells, is quite distinct. Even when the neighbouring epithelium is slightly green, as it sometimes is, we are unable to admit that the large secretion cells do not contain green granules. We have not only isolated the cells, but in teased fresh preparations have isolated the large green granules; and after disorganizing these by pressure, have seen the green substance of the granules collecting again to form green globules. There can be no doubt from our preparations that the green colour is in the large granular cells.

Our experience of the Marennes oysters is that the green colouration is disposed as follows:—All over both surfaces of the four gills; on the ribbed or grooved adjacent (adoral) surfaces of the four labial palps—that is, on the posterior faces of the anterior, and the anterior faces of the posterior palps (the other sides of the palps are very faintly coloured, if at all); occasionally the œsophagus to some extent (this is not at all constant); more frequently the rectum (but sometimes a brown rectum is found in a green-gilled oyster); the loop of intestine that coils around the stomach; and, lastly, the liver, to some extent.

When a piece of the gill in the living state is put under the microscope, the large green "macroblasts" are clearly visible. They are often arranged in longitudinal rows—parallel to the long axis of the filaments—packed closely together (Pl. IV., Fig. 3); sometimes they are a little spaced out, about their own diameter apart; in some places there are two rows on each filament in place of one, while in others they seem scattered quite irregularly. These cells give an eosinophilous reaction. In a previous paragraph (page 9) we have discussed the various forms of eosinophilous cells, and have shown that the regular grouping of these cells tends to indicate that

they are epithelial cells ("Beckerzellen"); we also showed that certain other eosinophilous cells in the epithelium were clearly wandering leucocytes, and of the same nature as the wandering cells met with in the connective tissue.

C. de Bruyne,* in his recent extensive work on Phagocytosis in Lamellibranchs, shows that in these molluscs there is a considerable exodus of leucocytes on the surface of all epithelia, both mucous and tegumentary. He considers that the diapedesis of leucocytes is always very important in the branchial epithelium, and becomes increased in the presence of any irritation. Our experiments described further on (p. 20) show that this is certainly the case in the oyster.

We regard the wandering cells with green granules, whether eosinophilous or not, which we find so plentifully in the epithelium of the gills and mantle (*see* Fig. 3), as leucocytes which are on their way to the surface, and as being of the same nature as the wandering cells we meet with in the connective tissue.

2. Roach River Green Oysters.—Certain parts of the estuaries of the Roach and the Crouch, in Essex, have long been known to produce green oysters; but these are usually regarded with so much suspicion in this country that, although healthy and delicately flavoured, they have to be sent abroad to find a market. We give a figure (Pl. IV. Fig. 9) of one of these oysters,† showing the rather pale blue-green colour of the gill. We find nothing abnormal or unhealthy in these oysters, and no unusual amount of copper or other metal in the green parts. The structure of the branchiæ and the cause of their colour are apparently the same as in the case of the Marennes oyster.

On the Essex coast, just as in the case of the French "claires," the Oyster-growers notice that immediately before the oysters become green, in autumn, a large amount of fine green weed or "moss" makes its appearance in the estuaries in question. We are indebted to Mr. E. Newman, manager of the Colne Oyster Company, for obtaining samples of this so-called "moss" for us to examine. We are now able to state that this green layer which appears as a coating on the mud ("London clay"), is a matted mass of minute Algæ, almost wholly Cyanophyceæ, such as *Microcoleus chthonoplastes*, *Lyngbya majuscula*, and *L. semiplena*. This is very similar, then, to the "verdure" of the French "claires" we have examined, except that in the latter several species of *Cladophora* were also abundant. (*See* Herdman "On Oyster Culture on the West Coast of France," Trans. Biol. Soc. Liverpool, vol. VIII., p. 113). In both cases many Diatoms are present, and these, along with the blue-green Cyanophyceæ, must form an important part of the oyster's food.

3. Green Dutch Oysters.—A large number of Dutch oysters from Nieuport, in Belgium, were sent over to us in January and February, 1897, by Dr. J. L. C. Pompe van Meerdervoort, who complained of a diffused greenness which appeared in October

* Contribution à l'Etude de la Phagocytose, Arch. de Biol. t. XIV., 1895; also t. XV., p. 270, 1898.

† From a batch kindly procured for us by Messrs. Cooke Bros., Oyster Merchants, West Mersea.

all over the bodies of his oysters, kept in artificial tanks, and which culminated in December in a more marked greening of the gills and labial palps, at the same time that the oysters became thin, watery, and transparent.

Our friend Dr. P. P. C. Hoek, of Helder, who happened to be working in our laboratory soon afterwards, examined these green oysters with us; and he has since written to us about a diffused greenness (which we find to be the same as that from Nieuport) which has broken out among the oysters stored on the farms at Bergen-op-Zoom, on the East Scheldt. Dr. Bottemanne, Inspector of Fisheries at Zeeland, has kindly sent us specimens of these green oysters from the Scheldt, and we find them to be, like those sent from Nieuport by Dr. Pompe van Meerdervoort, very thin oysters, in poor condition, with emaciated livers. Fig. 6 on Pl. IV. shows one of the largest of these Dutch oysters, and illustrates the characteristic colour of gill; but in most cases associated with this green colour we find a much smaller body, a shrunken liver, and a transparent watery mantle. Evidently mal-nutrition is one cause of the diffused pale greenness of these Dutch oysters. The reason of the mal-nutrition in these localities must be studied by some one on the spot; but it very probably lies in the practice of keeping the oysters in artificial cement tanks, into which water is only occasionally admitted. Even although the sea-water contains the usual amount of food, still Diatoms and other minute forms of life cannot be so abundant on the cement of the tank as they are on the mud forming the bottoms of estuaries, "claires," and pits.

Figs. 7 and 8 show, under a high power, sections of the external surface of the mantle, where (Fig. 7) a certain amount of green pigmentation of the epithelial cells is seen, in addition to several large coarsely-granular eosinophilous cells. There are also (Fig. 8), in the connective tissue under the epithelium, groups of large green globules, which are evidently MacMunn's "entero-chlorophyll." Fig. 10, showing a transverse section of the intestine unstained, is of considerable interest, as it shows that the green colour of the alimentary tract of this variety is due to a pigment in the epithelial cells. Thus the green colouration of this oyster is not similar to that found in the American oysters. Nor do we consider that it is identical with the green of the Marennes oyster, as the pigment is here (as our figures show) more generally diffused through the epithelium, and not aggregated in "macroblasts." The distribution and general appearance of these minute pigment granules recalls that of the green granules in the intestinal epithelium of *Chætop'erus*, as described and figured by Ray Lankester;* but the two pigments are quite distinct in their nature. It is evident, from recent work by Professor Lankester, Miss Newbigin, and others, that entirely distinct animal pigments may seem histologically very much alike in position and appearance.†

4. Falmouth Green Oysters.—From time to time in the past, since 1713, when a case of poisoning at the Hague‡ was attributed to oysters unlawfully coloured with

* Quart. Jour. Micros. Sci., vol. XL., p. 447.

† *See* Miss Newbigin's recent Paper in Quart. Jour. Micros. Sci., Nov. 1898.

‡ *See* O'Shaughnessy—Ann. and Mag. Nat. Hist., vol. XVIII., 1866.

copper to simulate the natural tint, green oysters from Falmouth and elsewhere in Cornwall have been suspected or accused of containing copper, derived from old mines or metalliferous strata in the sea. Quite recently Dr. Thorpe* has found on the average about 0.023 grains of copper oxide per oyster (in some cases much more) in specimens from Falmouth and Truro. On relaying, the green Cornish oyster gradually loses its colour, and is then found to contain only about 0.0062 grains of copper per oyster, which is practically the normal amount (0.4 milligramme) which we find in white "native" oysters from Whitstable.

Dr. Thorpe† says, "There is no question that the greenness of certain oysters, especially of those found in Falmouth and Truro waters, is due to copper. The colour, both in character and distribution is, however, quite different from that of the Marennes oyster." We can corroborate this. It is in effect what we have been stating for the last three years in our preliminary reports—that there are several kinds of green oysters; the "Huître de Marennes" is one of them, the copper-containing green oyster is another.

In the Falmouth oyster, containing an excessive amount of copper, we find that much of the copper is certainly mechanically attached to the surface of the body, and is in a form insoluble in water—probably as a basic carbonate. In addition to this, however, the Falmouth oyster may contain a much larger amount of copper in its tissues than does the normal colourless oyster. In these Falmouth oysters the cause of the green colour appears to be the same as in the green American oysters. Some of them also show (*see* Pl. VI., Fig. 8) a green heart of exactly the same nature as that of the green American oysters (Pl. VI., Figs. 3, 7, 8).

5. **Green American Oysters.**—This is the oyster at which we have chiefly worked. It is brought to Liverpool in large quantities, and is laid down on various parts of the neighbouring coast to be fattened and to be stored till required for the market. For some years it has been noticed that certain of these re-laid Americans may become green in the Autumn, the greenness being entirely different from that of the Marennes and other oysters we have examined, and consisting in a large increase of the granular green leucocytes of the blood in the heart, and in the sinuses and the tissues of the mantle, and also on the surface of the mantle, but very rarely in the gills. The result of this leucocytosis is that a more or less vivid green colouration is produced, which may be almost anywhere on the surface of the body, although it is most frequently over the liver, and the visceral (anterior) part of the body (*see* Pl. VI., Figs. 3—7, and 9). It may occur in patches or streaks, or very usually it is confined to the heart and blood channels, which are then engorged with a pale chalky-green mass of the granular leucocytes; in some cases, owing to the universal injection of the vessels and smaller blood spaces, the entire oyster has a greenish tinge (Fig. 6).

The oysters in which this massing of the green leucocytes occurs do not appear

* Local Govt. Bd. Report on Oyster Culture in relation to Disease, p. 105.
† *Nature* for Dec. 3rd, 1896, p. 107

especially if derived from organic matter, for they are quite free from any prejudicial influences traces of organic matter may exert, such as arise when volumetric or colorimetric methods are employed. In each determination the bodies or gills only of six or more oysters were carefully washed, dried between filter paper to remove as much adherent moisture as possible, and then carefully dried in porcelain dishes in the air-bath at 100° C. When this drying was as complete as possible, the oysters were heated in the air-bath until thoroughly carbonized, the carbon carefully burnt off over the free flame, and the residue finally ignited in a porcelain crucible. Special care was taken to exclude dust during both the drying and the ignition. The ash was then thoroughly extracted with a mixture of 25 c.c. hydrochloric acid and 25 c.c. sulphuric acid (1 : 2) on the water-bath, and the resulting solution filtered and concentrated. The residue was free from both copper and iron. The acid solution obtained was electrolysed for copper with the usual precautions, a spiral of fine platinum wire, weighing about 5 grms., being employed as the cathode. The wire was determined in the residual solution, after neutralization with ammonium hydrate, &c., acidifying with a few drops of oxalic acid solution, and boiling with ammonium oxalate. Four grms. of the oxalate were added in each case, the precipitated calcium oxalate (which is quite free from iron) filtered off and thoroughly washed, and the resulting solution electrolysed, the metallic iron being also deposited on a spiral of platinum wire. A blank experiment with all the reagents employed was made, and the amount of metal found (0.0002 grm. iron) deducted in each case. Also the deposited metal, both iron and copper, was dissolved off the electrode by acid, the solution obtained tested by the ordinary reagents, and the spiral re-weighed as a check upon the determinations, since the quantities found were extremely small.

I.—Determination of the Iron.

The following table* gives the results obtained, the quantities of iron found in green French as compared with white American oysters, three sets of gills being analyzed in each case. The figures show conclusively that there is more and not less iron in the gills of the white American oysters than in the French, and this irrespective of the basis on which the result is calculated. The ash is undoubtedly the most reliable factor to calculate on, provided the oysters are carefully washed before drying, which is always done: the result per set of gills (or oyster) is most in accord with this, and has the advantage of being an easy and in many respects useful basis.

---

* We are indebted for all the Tables in this section to the careful work of our colleague at Liverpool, Dr. C. A. Kohn, and we take this opportunity of thanking him most cordially for his kindness in helping us.

	Huîtres de Marennes.	American.
Gross body weight after drying between filter paper	3.8 grms.	6.5 grms.
Weight, dried at 100° C. for 6 hours	0.52 „	1.02 „
Weight of ash	0.0940 „	0.1140 „
Weight of iron found	0.0003 „	0.0008 „
Ratio of Iron found—		
(1) Calculated per set of gills	1 to	2.7
(2) „ on gross body weight	1 to	1.56
(3) „ on weight at 100° C.	1 to	1.36
(4) „ on ash	1 to	2.2

The relative quantities of iron present in the gills as compared with the rest of the body were next determined in French, Dutch, and American oysters. Six oysters, or the gills of six oysters, were analyzed in each case with the following results :—

Six Oysters.	Weight of Iron found, in Mgrme.		
	French.	Dutch.	American.
Gills	0.6	0.4	2.3
Bodies minus gills	1.2	1.5	1.7
Weight of ash of gills	0.1880	0.0217	0.0294
„ „ of bodies minus gills ...	0.5980	0.1125	0.1240
% iron in ash—gills	0.32	1.85	7.82
„ „ —bodies minus gills ...	0.20	1.33	1.37
Ratio of Iron in gills to Iron in rest of body—			
Calculated per oyster	1 : 2	1 : 3.75	1 : 0.74
„ on ash	1.6 : 1	1 : 4.1	5.7 : 1

Finally, the total iron in a number of kinds of oysters was determined in order to ascertain the normal quantity present. These data, which are tabulated below, show a fairly constant proportion of iron per oyster, from 0.15 to 0.36 mgrme., or from 0.18 to 0.65 per cent. on the ash.

Total Iron present in Oysters.

Kind of Oyster.	Number Analyzed.	Total Iron. Grme.	Weight of Ash.	Mgrme. Iron per Oyster.	Percentage Iron on Ash.
Huitres de Marennes... ...	6	0.0018	0.7860	0.30	0.23
Dutch	6	0.0009	0.1393	0.15	0.65
American	5	0.0018	0.2791	0.36	0.64
Colne	10	0.0020	1.0938	0.20	0.18
Deep Sea	2	0.0064	1.5017	0.32	0.43
Falmouth	6	0.0016	0.4534	0.27	0.35

In considering the variations in quantity, the very small amounts of metal present must be borne in mind.

From these figures in the above tables it is evident that there is not an excessive quantity of iron in the gills of the green oysters—the proportion of iron in the gills as compared with the rest of the body is somewhat more (1 : 2) than that found in Dutch oysters (1 : 3.7), but much less than in American oysters, which are white (1.4 : 1). The comparison is purposely made on the absolute quantity of metal in the gills and the rest of the body, as any other basis for calculation is fallacious.

Chatin and Muntz reckon on the weight of the dried organic matter present, but it was not found possible to get anything approaching constant weights in this way. This may to some extent account for the difference in our results, but we should also like to point out that although the ratio of iron in the gills and the rest of the body in green and in brown oysters is from 1 : 1.8 (feebly green) up to 1 : 2.3 (very green), they also instance white oysters with a proportion of 1 : 1.6.

It is certainly somewhat strange that they find more iron in the gills than in the rest of the body in all cases, which is not the case in our own experiments. But the quantities of metal present are so small that, since they do not state how many oysters were taken for analysis, and employ the permanganate method for the estimation of the iron, it is difficult to say what degree of absolute accuracy their results represent, and therefore to judge in how far their analytical data justify their conclusions.

From two points of view the above results show that the greenness of the gills of French oysters is certainly not due to iron :—

(1) Because the gills of the green oyster contain less iron than the rest of the body.

(2) Because the proportion of iron in the gills as compared with the rest of the body in white (American) oysters is greater than in the green.

That our method is reliable is shown by two determinations of the iron in the gills of American oysters, one giving 2.3 and the other 1.8 mgrme. of iron per six sets of gills. Variations in the size of the oysters will of course account for small differences, especially with the bodies.

It is evident from the last table that both the gills and the rest of the body of oysters contain normally (as would naturally be expected) a small quantity of iron, the gills having a somewhat larger amount in proportion to the total quantity of mineral matter present, but quite irrespective of the colour of the gills.

II.—Determination of the Copper.

Harless and Bibra* first showed that phosphate of copper was present in the blood of Molluscs (Cephalopoda), and Fredericq subsequently extended the observation† and proved that a certain small amount of copper is found normally in the blood of various Shellfish (both Crustaceans and Molluscs). It is apparently in combination with a proteid, to which Fredericq gave the name "hémocyanine."

The quantity thus present in oysters of different origin is fairly constant, as shown in the following table: it varies from 0.25 to 0.66 mgrme. per oyster, or from 0.30 to 1.18 per cent. on the ash:—

Kind of Oyster.	Number Analyzed.	Total Copper. Grme.	Weight of Ash.	Copper per Oyster. Mgrme.	Percentage Copper on Ash.
Huîtres de Marennes ...	6	0.0024	0.7860	0.40	0.30
Dutch	6	0.0015	0.1393	0.25	1.08
American	5	0.0033	0.2791	0.66	1.18
Colne River	10	0.0036	1.0938	0.36	0.33
Deep Sea (very large) ...	2	0.0069	1.5017	0.34	0.46

For copper, then, 0.4 mgrme. per oyster may be taken as an average, a quantity slightly greater than the average iron (0.26 mgrme.). The calculated percentages on the ash show greater variations, due to the very considerable differences in the total quantities of mineral salts present; and it is very possibly to this last factor that the well-known differences in taste of the various kinds of oysters is at any rate in part due. Certainly the minute quantities of copper and iron present cannot account for the flavour, as has been sometimes suggested.

The copper was also determined in the gills, and in the bodies minus gills, of French, Dutch, and American oysters, with the following results:—

Six Oysters.	French "Huîtres de Marennes" (green).	Dutch (slightly green).	American (white).
Gills only	Trace.	0.8	1.7
Bodies minus gills...	2.4 mgrme.	1.4	3.3

* Müller's Archiv, 1847. † Rech. s. l. Physiologie du Poulpe, Arch. Zool. Exper., t. VII. (1878).

These results show that copper is present normally in both green and in white oysters in small quantity, but that the greenness of the gills of French oysters is certainly not due to this metal—in fact they contain only the merest trace.

Experiments which we have made on the feeding of oysters with very dilute saline solutions of iron and of copper salts, entirely confirm these analytical data. Beyond a certain amount of post-mortem green staining, the oysters did not acquire any green colour. We give the details of these feeding experiments further on.

There are, however, some green oysters (not the "Huîtres de Marennes") which, as we have already stated, contain much more copper than those analyzed above. They are probably under abnormal conditions. Such cases are certain Falmouth oysters, and the American oysters with the green leucocytosis.

Dr. Thorpe has shown that some green oysters picked out by Dr. Bulstrode at Falmouth and Truro contained as much as 0.02 grains per oyster on the average—some contained much more; while we find that the green American oyster from Fleetwood, where the greenness is due to the leucocytes, has on the average 0.0229 grains of copper per oyster—between three and four times the normal amount.

We have been able to obtain some of these green oysters from Falmouth for examination and analysis. Six Falmouth oysters (*O. edulis*), the bodies of two of which were of a distinct arsenic green colour, were dried at 100° C. and then digested with water, and subsequently with dilute hydrochloric acid. The extract contained about half the total copper present, showing that the metal is partially, at any rate, mechanically retained in or on the body of the oyster,* probably as a basic carbonate.

The analytical results were as follows:—

Six Oysters.	Copper.	Iron.	Weight of Ash.	Mgrme. Copper per Oyster.	Mgrme. Iron per Oyster.	Per cent. Copper on Ash.	Per cent. Iron on Ash.
Extract with dilute acid	0.0097	0.0024	0.2272	1.62	0.40	4.22	1.06
Oysters	0.0114	0.0016	0.4534	1.90	0.27	2.51	0.35
Total	0.0211	0.0040	0.6806	3.52	0.67	3.10	0.59

The total copper present here is almost nine times the normal quantity, and about half of this is easily removed by dilute acid. It is quite likely that the remainder of the excess is partially or wholly simply entangled in the food passages of the oyster.

The occurrence of copper in oysters, under such conditions, in some parts of Cornwall is due to the locality, and might quite possibly attain injurious proportions. The oysters analyzed were obtained from a creek which is locally supposed to bring

* Dr. Thorpe, in a letter to *Nature* (1896, p. 107), referring to the large proportion of copper in these Falmouth oysters, speaks of it as "obviously caused by the mechanical retention of cupriferous particles."

down copper from old mines, and the mud of which was found by Thorpe to contain 0.148 per cent. of copper. Normal sea-water contains such an excessively small quantity of copper that it was not found possible by Dr. Kohn to detect its presence, even electrolytically, in a litre of sea-water, after concentration.

Taking, finally, the green leucocytosis found in some American oysters, the six greenest and six whitest of 120 of these oysters, as re-laid on our coast, were chosen for analysis; also a quantity of the greenest portions of the greenest oysters was selected from another batch, and compared with the corresponding portions of the whitest oysters. The iron was not determined in the latter comparison, owing to the possible contamination by that metal in the cutting.

The following were the results obtained :—

Oysters.	Copper.	Iron.	Ash.	Mgrme. Copper per Oyster.	Mgrme. Iron per Oyster.	Per cent. Copper on Ash.	Per cent. Iron on Ash.
Green	0.0158	0.0091	1.1450	2.63	1.52	1.38	0.79
White	0.0042	0.0036	1.0948	0.70	0.60	0.38	0.33
Greenest parts ...	0.0033	—	0.0780	—	—	4.23	—
Whitest parts ...	0.0009	—	0.0452	—	—	1.99	—

The excessive quantity of copper in the selected green oysters is 3.75 times that in the white ones, calculated per oyster, and 3.63 times calculated on the ash. In the selected parts the total copper present calculated on the ash is high in both cases, and the green parts again show a marked excess in the proportion of 2.1 to 1. The copper and iron in the white specimens are about normal, but the increased quantity of iron in the green is marked, being 2.5 times that of the former. Still there is relatively a large excess of copper as compared with iron in the green oysters, as is evident from the analyses, the ratio being 1.1 : 1 for the white, and 1.8 : 1 for the green.

It is to be concluded, therefore, that the green colour of these diseased American oysters is coincident with the distribution of the excessive quantity of copper present, and that the copper is in consequence to be regarded as the cause of the colour. The histo-chemical investigations given further on confirm this conclusion.

Manganese was also found by Dr. Kohn to be present in several of the kinds of oysters analyzed. Its detection is readily effected in the electrolytic method of analysis, as it separates at the anode as peroxide. Colne oysters contain 0.14 mgrme. per oyster, a quantity rather smaller than the amount of iron present.

We must now turn to Dr. D. Carazzi's recent papers, in which he tries to show that the green colouration in oysters is in all cases a healthy condition, caused by the absorption of iron as a nutrient material from the surrounding medium by means of the surface epithelial cells, such as those of the branchiæ and palps. A good deal that is

stated by Carazzi is quite correct, but these are points which were known before. The novel statements are, we believe, entirely erroneous, and are the results either of mistaken observations, or inferences from observations.

He rightly states that the Marennes green oysters may retain their colour for weeks or even months when taken to other waters in which greening does not take place. It is true of our green Marennes oysters, kept for weeks practically without food in tanks in the laboratory. This fact is well known, and has been mentioned by various writers from Bouchon-Brandely, in 1871, onwards; but does it not tell strongly against Carazzi's own view that the green substance is a nutrient material on its way from the surface epithelium to the liver? Is it at all likely that, if the green "Marennin" is a nutrient material in process of metabolism, it will remain for months in the superficial tissue (which by Carazzi's hypothesis has just absorbed it) after the source of supply has been removed? That is very improbable, even admitting Carazzi's statement as to the distribution of the iron-containing Marennin in the superficial cells to be correct; but we do not admit it. As we have shown above, we have abundant evidence that the green granules are constantly in the "macroblasts," and comparatively rarely in the ciliated epithelium. In fact, in all essential points of structure—the details of which need not be gone over again—we agree with Lankester, Pelseneer, J. Chatin, and De Bruyne, and differ from Carazzi in his criticism of these former authors.

Moreover, all the observations made by ourselves, and those recorded by others, on the West Coast of France are in accord with the view that the "greening" depends upon microscopic food-organisms in the sea-water. Bornet and Ad. Chatin showed, in 1894, that the greening comes on suddenly in parks at Sables d'Olonne, where it was not present before. The greening, again, only takes place at a certain time of year, usually autumn, which agrees with the periodic distribution of minute organisms. Bouchon-Brandely says that at Marennes it is a common observation that the oysters only become green when the water in the "claires" (ponds) has become green with microscopic plants. It is known at La Tremblade that when a claire is in the greening condition it must not be emptied, as a change of the water might deprive it of its greening properties. Moreover, the greening must be due to the contents of the water, since in the Island of Cuhan (La Trinité River) Dr. Gressy has successfully reared green oysters in basins excavated in the solid rock.

We must call attention to the way in which Carazzi has peremptorily denied * or misrepresented the results of some of the many investigators of molluscan anatomy and physiology with whom he does not agree. For example, he says of Ryder's statement that the ventricular part of the heart may be green, "he is certainly mistaken," &c. We are able, however, to state that Ryder was perfectly correct, and that it is

* It is perhaps sufficient, as an example of Carazzi's style, to state that after alluding to the work of Lankester, Pelseneer, and others, he makes the sweeping assertion :—"Tutto quel ch' è stato detto finora sulle ostriche verdi è completamente sbagliato, ed è la conseguenza di osservazioni o infondate o malamente interpretate."

Carazzi who is mistaken, as we have found exactly the condition that Ryder describes in American oysters (*see* Pl. VI., Fig. 7).

In connection with this we may state that we have had the advantage of discussing very fully the iron and copper reactions, and Carazzi's statements and criticisms, with Professor A. B. Macallum, of Toronto, recently in our laboratory. Macallum, who is the author of the hæmatoxylin method of demonstrating iron in tissue-cells, agrees with us entirely, and wishes to repudiate Carazzi's remarks in regard to the iron reaction.

Finding that we considered it necessary to take notice of Carazzi's papers, Dr. Macallum has asked us to insert the following note, which he had already written out for publication, as a statement of the method in which Carazzi has misinterpreted and misapplied his (Macallum's) and Zaleski's reactions :—

> "In his reference to the literature Carazzi has, in some cases, been particularly inexact. He attributes to Macallum a method of liberating the organic iron of cellular elements (treatment with Bunge's fluid), in order to demonstrate it in the usual way, which that observer not only did not use, but also expressly describes as unsuitable for the purpose. Carazzi obtained negative results only with this method, the only one which he used, and yet he ventures on the basis of results so obtained to deny the correctness of Macallum's observations. In regard to Zaleski, Carazzi is almost equally inaccurate. Zaleski states specially that if the isolated liver-cells are extracted with physiological salt solution, and the residue subjected to artificial digestion after thorough peptonization, nucleins remain in which the iron can be shown 'nicht aber mehr durch die unmittelbare Anwendung der Eisenreagentien,' but after complete incineration only. One nuclein compound, not obtained in this way, did indeed give an immediate reaction for iron; but Carazzi practically states that all the iron-holding nucleins isolated by Zaleski reacted immediately for iron, and then claims that this accounts for all the iron found in the nucleus by Macallum. An inexact knowledge of the literature on a subject is bad, but a criticism based on it is worse—it is mischievous."

IX.—MICRO-CHEMISTRY OF THE GREEN OYSTERS.

(See Plates VII. and VIII.)

The following are the details of the histo-chemical investigation of the pigment of the green American oysters with the leucocytosis. The green pigment is insoluble in boiling alcohol, ether, chloroform, xylol, and other fat solvents; it is soluble in dilute acids and alkalies. The addition of potassic ferrocyanide to sections containing the green colouring matter, or to the leucocytes themselves, gives a red reaction, indicating the presence of copper; but the reaction can be most readily obtained by the addition of a small quantity of 5 per cent. hydrochloric acid to the potassic ferrocyanide. Ammonium hydrogen sulphide gives also an immediate reaction with the green pigment. Ammonia strikes a beautiful blue wherever there is green in marked quantity. It was also found that pure hæmatoxylin is an extremely delicate test, giving an immediate blue reaction in exceedingly dilute solution. Previous treatment of the green colouring matter by 3 per cent. nitric acid in alcohol prevented these reactions, and subsequent treatment with acidulated potassic ferrocyanide resulted in a very faint Prussian blue colouration of the tissue generally (marking out especially the nuclear zone in any epithelium present). We concluded that there was *no organic iron* present in the leucocytes, that the leucocytes which form the green patches contain a considerable quantity of copper, and that, just as in the case of iron, as shown by Professor Macallum, pure hæmatoxylin is a most delicate test for copper, but that great care must be taken to ascertain by other reagents which of the two metals is present.

Very numerous tests were made with the blood obtained from white oysters, and micro-chemical reactions revealed in some instances faint traces of copper. Hæmocyanin has been described in the blood of various Molluscs, and apparently in the blood of the oyster. We have examined numerous samples of blood taken from the white oyster, but have failed to get any blue colouration on exposure to air. In the green oysters a very faint blue colour has been noticed in some cases on exposing the blood to air.

To demonstrate the presence of copper in the green leucocytes collected in the vessels, the tissues may be examined fresh or after hardening.

Fresh Blood.—By means of a fine pipette the green corpuscles are removed from the heart or other blood space they have collected in, and placed on a cover slip and allowed to dry. The dried film, which has a green tint, is then treated with the clear dilute hæmatoxylin solution, and at once assumes a deep violet colour. Similarly, with

potassic ferrocyanide, after previous immersion in very dilute acid, a distinct reddish colouration is obtained, visible to the naked eye. Fig. 3 on Pl. VII. shows part of such a preparation under the microscope. Colourless corpuscles obtained from oysters which are not green do not give these reactions. The best preparations of the copper-containing leucocytes are obtained by treating freshly-teased vessels, or sections of vessels, after imbedding in paraffin, with the various reagents (*see* Pl. VII., Figs. 1, and 4—9). The leucocytes in bulk then give well-marked reactions; in many of the cells the reactions can be plainly seen, the granules in them assuming a reddish tint in the case of potassic ferrocyanide, or violet with hæmatoxylin.

Vessels and Heart.—In well-marked cases of leucocytosis the green heart (Pl. VII., Fig. 2, *b*) may be removed from the oyster, treated first with dilute acid, and then with potassic ferrocyanide, when a brown colouration is readily obtained; the organ may then be dehydrated, passed through oil of cloves, and mounted in Canada balsam. Similarly the vessels may be stripped off from the mantle and treated as above—such specimens are seen in Figs. 1 and 4. The most instructive preparations are, however, obtained by first hardening the green oysters. Figs. 5—9 on Pl. VII., and Figs. 5 and 6 on Pl. VIII., show a series of sections through the body of the oyster, and passing through one of the larger pallial sinuses close to the mantle edge, which in this case was engorged with green leucocytes. One section, Fig. 7, has been treated with potassic ferrocyanide; Fig. 9 has been stained with the colourless solution of hæmatoxylin; Fig. 8 has been treated with ammonium hydrogen sulphide; and Fig. 6 is an untreated section which plainly shows the green colour. Similar reactions are demonstrated in our series of sections (which it seems unnecessary to reproduce, as they show the same colours as Figs. 6—9) passing through the auricles and ventricle; the conjoined auricles, it will be observed, have normally a dark brown colouration, and do not give any change, the ventricle containing the leucocytes only reacting.

Under high magnification (*see* Pl. VIII.) the best pictures* are yielded by the hæmatoxylin-treated sections. Wherever there are copper-containing leucocytes, these stain immediately with the reagent, and by their colour stand out in marked contrast with the surrounding colourless tissue. This is well illustrated in Pl. VIII., Figs. 1 to 4. In many of the sections we found that the large eosinophilous cells in the mantle of the green oysters gave a strong reaction with hæmatoxylin; this was the case in the oyster from which Pl. V., Fig. 8, was made. That eosinophilous cells should give such a reaction is remarkable, and is to be explained by the fact that in some cases these cells contain copper.

* In these and the other figures illustrating the micro-chemical reactions, we have given the colour effects, distribution of granules, and shades, but have not represented the further histological details which these methods were not intended to demonstrate.

X.—EXPERIMENTS IN FEEDING WITH METALLIC SALTS.

With the object of trying to produce in the laboratory results similar to those observed in Nature, and with the view of testing the part which the absorption of metallic salts might play in colouring the oyster, and in possibly rendering it unfit for human food, we have made, during the last couple of years, a large number of experiments by keeping oysters in tanks in which definite quantities of various salts of iron and copper had been added to the sea-water.*

In all cases we first of all ascertained that our oysters, both Americans and Natives, were healthy and colourless, so far as we could judge by observing them through the opening between the shells when in the expanded condition. We could make sure at any rate that the mantle and the gills were not green, and we always opened a few specimens of the same batch as a control.

In the first place, we tried the effect of pieces of copper, copper filings, and copper dust lying in the bottom of the aquarium; and similarly, of steel filings, old rusty nails, and other fragments of iron. We also kept oysters for some time in an old copper vessel, and along with copper pyrites and other ores of copper. None of these gave any definite result.

We then, with Dr. Kohn's assistance, tried measured quantities and strengths of various metallic salts. On Feb. 5th, 1896, we added 0.2 per cent. of sulphate of copper to 10 litres of sea-water in which a few oysters were placed; while another set of oysters had the same quantity of ferric ammonium citrate added to their 10 litres of water. From these we got no definite results. The oysters lived better in the iron water than in the copper. The only staining was a deposition on the shell and in the mucus of the mantle edge, and some *post-mortem* colouration in the case of those that died.

On Feb. 27th we started three colourless American oysters in each of three aquaria containing insoluble salts, viz., sulphide of iron, ferric hydrate, and copper oxide, in each case 50 grains being added to one gallon of sea-water. By March 5th, those in the copper oxide fluid were evidently sickly, while the others seemed healthy. On March 12th, those in the copper oxide no longer reacted under stimulation, and were evidently just dead. On being opened, they had all the appearance of ordinary yellow oysters, the gills and palps were normal, and there was no trace of staining in any part. On March 21st, one from each of the other aquaria was opened. They were

* Recently Carazzi has stated that oysters fed with similar dilute iron solutions acquire a pale yellowish colour in certain parts (branchial epithelium and the œsophageal mucous membrane), and that in these parts microscopic tests show the presence of granules of iron. The actual meaning of these results can hardly be recognised without comparative and quantitative data.

found to be alive and healthy, with the heart beating actively, and no green or brown staining in any part. The rest were opened on March 26th, and found to be in the same healthy condition.

Dr. Kohn then made us up some soluble salts, and on May 27th two batches of oysters were put in tanks of sea-water containing a small amount of chloride of copper. On June 4th, three were found to be dead, and on examining these one had a slight greenish tinge over the viscera and a green spot in its heart, and the second had a good deal of green over the right side of its mantle, and many of its vessels were engorged with pale green leucocytes. The third specimen was not green, and in none of them was there any greenness in the gills. Twenty of these same oysters, opened at the beginning of the experiment, showed only one slightly green specimen. On June 5th, another specimen from the copper solution showed a greenish mantle, and the following day the remaining five oysters, when opened, were found to be all more or less tinged with green—as follows :—

A. Green on mantle over viscera, and slightly on mantle over gills. Heart very green and chalky.

B. Green on mantle over gills, and heart slightly so.

C. Green on mantle over gills, and heart very green.

D. Very green on mantle over gills, and slightly over viscera. Heart pea-green.

E. Slightly green on mantle over gills and over viscera. Heart pale chalky green.

The gills themselves were scarcely affected in any case. In the case of the mantle, the staining was not on the surface, but in the leucocytes in the tissues and blood spaces.

A second batch started on June 5th, in the same soluble copper salt, were opened on June 11th and 12th, when it was found that all had their hearts more or less green, and the mantle over the viscera stained, and having blood-vessels engorged with green leucocytes. Most of them had the mantle over the gills stained of a greyish-blue colour. The gills themselves were not affected in any case. A control batch of four of the same oysters kept in ordinary sea-water showed, however, one heart very green and the other three slightly so. One had the vessels over the viscera engorged, and there was a little green staining in the mantle of all.

It was evident, then, that these oysters were becoming green to some extent, quite apart from any action of the copper, but those in the copper solution were decidedly greener than the control oysters. On June 12th, some further experimental batches were started, (*A*) in ordinary sea-water, (*B*) in a solution of the same copper salt in which 10 c.c. was added to the gallon, and (*C*) another in which 1 c.c. was added per gallon. On June 18th, (*A*) showed four normal oysters, and one specimen a little green in the heart ; (*B*) showed one specimen normal, and four all somewhat green ; (*C*) showed three with no green, two with engorged vessels containing green leucocytes, and one of them with a green heart.

These are samples of a large number of similar experiments* made with different salts of copper and iron, soluble and insoluble, and of various degrees of strength, which give, on the whole, the following results :—

1. In no case was any greening at all comparable with that of the Marennes oysters produced.
2. The insoluble salts which lay as a deposit at the bottom of the aquarium seemed to produce little, if any, effect.
3. The soluble salts of iron either produced no effect, or had a favourable effect, the oysters living rather better in these solutions than in ordinary sea-water. They gave rise to no greening.
4. The very weak soluble copper-salts had little, if any, effect; while the stronger copper-salts had always an unfavourable effect upon the life of the oyster, causing death sooner or later; and in most cases producing more or less of that unhealthy green leucocytosis which we had found in various kinds of oysters bedded around our coast, and which Ryder evidently found amongst oysters on the American coast.

* Since this was written we have carried out (October to December, 1898) a series of further experiments at the Lancashire Sea Fisheries Hatchery at Piel Island, in the Barrow Channel. The oysters were kept in tanks through which a constant current of fresh sea-water was running. To this current measured quantities of dilute copper and iron salts were added drop by drop, in such a way as to mix thoroughly with the sea-water before reaching the oysters. We have examined these oysters, and find that those that have been for from one to three weeks in the current containing the copper solution, show evidence of having more than the normal amount of copper in their bodies, but still there was no apparent greening. Those in the iron solution were unaffected.

These experiments are being continued for us by Mr. Andrew Scott, Resident Assistant at the Piel Hatchery, and will be shortly extended to mussels and cockles, which, we find, are also liable to become green.

XI.—ON THE CONNECTION BETWEEN OYSTERS AND INFECTION.

From the earliest times more or less well grounded suspicion has been cast from time to time upon shellfish—chiefly Oysters and Mussels—as being the cause of outbreaks of disease amongst consumers, such as :—1st. Cases of sudden poisoning due to the presence of putrefactive products in the shellfish ; and 2nd. Diseases due to a specific micro-organism, where there is a period of incubation, and where therefore a considerable interval has elapsed between the infection and the actual illness. In the latter case it is obviously much more difficult to determine with certainty the source from which the disease germ has entered the body ; and although many positive assertions have appeared of late years attributing outbreaks of enteric or typhoid fever to the consumption of oysters, still it must be pointed out that the connection between the two, although quite likely, has not yet been scientifically proved, and is only at present more or less of a possibility, or, at most, probability.

During the past three years we have been making a number of observations and experiments, both in our Liverpool laboratories and at the Port Erin Biological Station, upon the conditions under which oysters live healthily, and upon the possibility, or even in some cases the probability, of their being the carriers of disease germs. During that period much public interest and apprehension has been raised by several recent outbreaks of typhoid popularly attributed to oysters, such as that of the Stirling Ball in October, 1895 ; that of the Wesleyan University in Connecticut, investigated by Professor H. W. Conn in 1894 ; and that in the south of France attributed to Cette oysters, reported upon by Dr. Chantemesse in 1896. It was obvious from these, and from numerous other outbreaks of typhoid attributed to eating oysters, that the conditions under which the shellfish were liable to become contaminated with sewage, and so be the carriers of pathogenic organisms, required investigation. The following is a brief account of our experiments and observations.

A. The objects we had in view in entering on the investigation were as follows :—

1. To determine the conditions of life and health and growth of the oyster by keeping samples in sea-waters of different composition ; *e.g.*, it is a matter of discussion amongst practical ostreiculturists as to what specific gravity or salinity of water, and what amount of lime, are best for the due proportionate growth of both shell and body.

2. To determine the effect of feeding oysters on various substances—both natural food, such as diatoms, and artificial food, such as oatmeal. Here, again, there is a want of agreement at present as to the benefit or otherwise of feeding oysters in captivity.

(*d*) A series of oysters weighed approximately, and fed upon the following substances, viz :—(1) Oatmeal, (2) Flour, (3) Sugar, (4) Broth, (5) Living Protophyta (Diatoms, Desmids, Algæ), (6) Living Protozoa (Infusoria, &c.), (7) Earth.

In this series of experiments the oysters were fed every morning, and the water was aërated but not changed (evaporation was compensated for by the addition of a little tap water as required). The oysters were weighed from time to time, and observations made upon the apparently harmful or beneficial effects of the above methods of treatment.

(*e*) A series of oysters placed in sea-water to which was added daily :—

(1) Healthy fæcal matter.

(2) Typhoid fæcal matter.

(3) Pure cultivations of the typhoid bacillus.

The oysters were carefully examined to determine their condition, with special reference to condition of branchiæ, alimentary canal, adductor muscle, liver, and viscera generally.

The contents of the rectum, as well as the water in the pallial cavity, were subjected to bacteriological analysis, to determine the number of micro-organisms present, per cubic cm., as well as the identity of the typhoid or other pathogenic organisms.

C. The following is a summary of the results obtained :—

I. The beneficial effects of aëration :—

(*a*) By the addition of air only,

(*b*) By change of water :

pointing to the conclusion that the laying down of oysters in localities where there is a good change of water, by tidal current or otherwise, should be beneficial.

II. The diverse results obtained by feeding upon various substances, amongst which the following may be noted :—The exceedingly harmful action of sugar, which caused the oysters to decrease in weight and then die ; whilst the other substances detailed above enabled them to maintain their weight or increase. The oysters thrive best upon the living protophyta and protozoa. Those fed upon oatmeal and flour after a time sickened, and eventually died. Some were fed upon quantities of a minute green alga (*Microcystis*), which we happen to have in abundance in a fountain. This did not result in any greening of any part of the oyster.

III. The deleterious effect of stagnation, owing to the collection of excretory products, growth of micro-organisms, and formation of scum upon the surface of the water.

IV. The toleration of sewage, &c. It was found that oysters could, up to a certain point, render sewage-contaminated water clear, and that they could live for a prolonged period in water rendered completely opaque by the addition of fæcal matter ; that the fæcal matter obtained from cases of typhoid was more inimical than that

obtained from healthy subjects; and that there was considerable toleration to peptonized broth.

V. The infection of the oyster by micro-organisms.

The results of the bacteriological examination of the pallial cavity of the oyster, and of the contents of the rectum, showed that in the case of those laid down in the open water of the Bay the colonies present were especially small in number, whilst in those laid down in proximity to the drain-pipe the number was enormous (*e.g.*, 17,000, in one typical culture, as against 10 in the former case). It was found that more organisms were present in the pallial cavity than in the rectum. In the case of the oysters grown in water infected with the *Bacillus typhosus*, it was found that there was no apparent increase of the organisms, but that they could still be identified in cultures taken from the water of the pallial cavity and rectum fourteen days after infection.

It was found that the typhoid bacillus would not flourish in clean sea-water, and our experiments seemed to show that it decreased in numbers in its passage along the alimentary canal of the oyster. It would seem probable, therefore, that by methods similar to those employed in the "Bassins de dégorgement" of the French ostrei-culturists, where the oysters are carefully subjected to a natural process of cleansing, oysters previously contaminated with sewage could be freed of pathogenic organisms or their products without spoiling the oyster for the market.

It need scarcely be pointed out that if it becomes possible thus to cleanse infected or suspected oysters by a simple mode of treatment which will render them innocuous, a great boon will have been conferred upon both the oyster trade and the oyster-consuming public.

The remaining sections of this memoir deal with further points and investigations in connection with the infection and disinfection of the oyster and other shellfish.

XII.—CONDITION OF OYSTER BEDS AND LAYINGS.

It has been known for some time that many of the places in estuaries or harbours where oysters are laid down to fatten, or to await their market, have been kept in a most insanitary condition, either because the water as a whole is polluted, or because the opening of some drain-pipe is in such close proximity that the discharge from the drain is liable to pass over the shellfish. We showed in 1895, in our preliminary paper, that oysters have a very remarkable power of withstanding organic impurities in the water, and can continue to live in water much polluted with sewage. In fact they are able, if kept sufficiently aërated, to render water almost opaque with sewage clear in a wonderfully short time.

As the result of our observations on oyster-beds, and our experiments on the kinds of water in which oysters will live, we recommended, in January, 1896,*

"1st, A strict examination of all grounds upon which oysters are grown or bedded so as to ensure their freedom from sewage; and

2nd, If practicable, the use of 'dégorgeoirs' or disgorging tanks, in which the oysters should be placed for a short time before they are sent to the consumer."

Towards the end of the same year, the Local Government Board Report on Oyster culture in relation to Disease, containing sections by Sir Richard T. Thorne, Dr. H. T. Bulstrode, and Professor E. Klein, appeared, giving detailed statements as to the insanitary conditions under which oysters are grown or kept on various parts of our coasts. Dr. Bulstrode has shown conclusively, by his descriptions and plans, what was known previously only to those who had taken the trouble to look into the matter, that while some of the localities from which our oysters come to market are excellently suited for the purpose and free from reproach, other "layings" are in a disgraceful condition of sewage contamination. That is undoubted; but as to the further question, whether the typhoid organism, if it gains access to the body of the oyster from sewage, finds itself in a suitable medium for continued life and multiplication, it is difficult to arrive at any definite conclusion. Professor Klein's experiments, like our own (see below, p. 45), are not sufficiently extended to be positive upon this point. There is, however, a marked difference between the cultures obtained from oysters taken from a contaminated source and those from pure water free from all possible sewage pollution. The body of the oyster is probably not a favourable medium for the growth of pathogenic organisms, the risk of infection is probably therefore never great, and if a little more care were exercised there would be practically no danger at all.

* Report to the Lancashire Sea Fisheries Committee: Trans. Biol. Soc. Liverpool. Vol. X., p. 174.

What is really required is—

1st. That some central, or local, Authority should inspect and license the oyster beds and layings, and that without such license, no oysters should be sold*; and

2nd. That all those who cultivate and those who sell oysters should exercise ordinary common sense, and elementary notions of decent cleanliness, in their responsible position as purveyors of an important and nutritious article of diet.

Mr. C. E. Fryer, in the recently published 12th Annual Report of the Sea Fisheries Inspectors for England and Wales, discusses this question, and points out the difficulties in the way of preventing polluted oysters being shipped from unlicensed to licensed beds, and in regulating the importation of foreign oysters. However, as he himself states, these difficulties are not insurmountable.

We quite agree with Mr. Fryer's further remark, that whatever precautions and regulations are adopted for preventing the distribution of contaminated shellfish, they ought to include measures for duly apportioning among the various parties concerned (such as those interested in the drains as well as those interested in the oysters) the responsibility for the contamination.

* Since the above was written, an Oyster Bill has been laid before Parliament, which, although capable of improvement in some details, does much to meet the present difficulties.

XIII.—THE BACTERIOLOGY OF THE OYSTER AND OTHER SHELLFISH.

Our investigations under this head have been made in the following directions:—

1. *The Identification and Differentiation of the Bacillus typhosus and B. coli communis.*
2. *The action of sea-water upon the growth of the Bacillus typhosus.*
3. *The infection of the oyster with the Bacillus typhosus.*
4. *The bacteria present in the alimentary canal of the oyster and other shellfish.*
5. *The occurrence of Bacillus enteritidis sporogenes in shellfish.*

In September, 1896, an abstract of our results was published at the Liverpool Meeting of the British Association, and shortly afterwards the Local Government Board Report upon "Oyster Culture in Relation to Disease" appeared (November, 1896). The report was a very full one, and was furnished by Dr. Bulstrode and Professor Klein, and summarised by Sir Richard Thorne Thorne. The ground covered by Professor Klein and by ourselves was much the same, and our results approximated to his. Since then we have continued the work, and have repeated and extended our observations, of which the following are the more important.

I.—The Identification and Differentiation of Bacillus typhosus and B. coli communis.

We have systematically tested the majority of the chief differential reactions upon samples of *B. typhosus* and *B. coli* obtained from numerous sources, and have in all cases found unmistakable differences between the two bacilli.

Table showing Differences of Reaction.

Source.	Fermentation, Glucose Gelatine.	Indol Reaction.	Coagulation.	Potassium Iodide Potato Gelatine.
I.—B. typhosus.				
1 Institut Pasteur	None.	None.	None.	Very small growth.
2 From spleen of Typhoid patient	,,	,,	,,	,,
3 Prof. Delépine	,,	,,	,,	,,
4 Prof. Wright (Netley)... ...	,,	Slight trace.	Clot slowly formed.	,,
5	,,	None.	None.	,,
6 Dr. Sims Woodhead ...	,,	,,	,,	,,
7	,,	,,	,,	,,
8	,,	,,	,,	,,
9 Dr. Kanthack	,,	,,	,,	,,
10 Institute of Preventive Medicine	,,	,,	,,	,,
II.—B. coli.				
1 Institut Pasteur	Well marked.	Marked pink.	Marked.	Growth abundant.
2 Prof. Delépine	,,	Slight pink.	,,	,,
3 Prof. Wright	,,	Pink.	,,	,,
4	,,	Slight pink.	,,	,,
5	,,	Marked pink.	,,	,,
6	,,	Pink.	,,	,,
7 Dr. Sims Woodhead ...	,,	,,	,,	,,
8	,,	,,	,,	,,
9	,,	,,	,,	,,
10	,,	,,	,,	,,
11	,,	,,	,,	,,
12 Dr. Kanthack	,,	Slight pink.	,,	,,
13	,,	Absent.	,,	,,
14 Institute of Preventive Medicine	,,	,,	,,	,,

Summary of Constancy or Variability of Reactions.

B. typhosus.—(1) Fermentation test; no gas formation. (2) Indol reaction; slight indication in one case. (3) Milk coagulation; slight clot in one case. (4) Potassic iodide potato gelatine; characteristic invariably. (5) Potato; constant with usual precautions. (6) Reaction in gelatine; marked differences of rate of diffusion.

B. coli.—(1) Fermentation; rate of gas formation variable. (2) Indol reaction; not constant. (3) Milk coagulation; constant. (4) Potassic iodide potato gelatine; abundant growth. (5) Behaviour in gelatine; diffusion very variable, in many cases less rapid than with *B. typhosus*. (6) Motility; very variable.

This preliminary investigation proved of great service in familiarizing ourselves with the appearances of typhoid and colon bacilli, and prepared the way for the subsequent investigations.

II.—The Action of Sea-Water upon the Growth of the B. typhosus.

Eight experiments were made to ascertain whether the *B. typhosus* was capable of multiplying in sea-water. The subject was naturally one of very considerable importance in connection with the liability of oysters to infection when grown in sewage-contaminated water. Our experiments showed that sea-water was inimical to the growth of the *B. typhosus*.

When a large number of bacilli are added to the water, their presence may be demonstrated longer than in cases where smaller quantities are used. Fourteen days would appear to be the average duration in sea-water incubated at 35° C.; whilst, kept in the cold, their presence was demonstrated, as will be seen by experiment VII., on the twenty-first day. There appears to be no initial or subsequent multiplication of the bacilli. Between forty and seventy hours after infection there is less decrease than at other periods; but there is no evidence of increase in numbers of the bacilli, when grown in sea-water, either when incubated or at ordinary temperatures. Dr. Klein, in his Report, states that in no instance did he find evidence of multiplication of the bacilli in the sea-water. He was able to recover the bacillus up to the fourth week. In 1889, Giaxa made a series of observations upon the vitality of the *B. typhosus* in sterilized and non-sterilized sea-water, and showed that it was present in the latter up to the ninth day, and in the former to the twenty-fifth. In this connection may be taken the observation of Frankland and Ward,* that a 3 per cent. salt solution most prejudicially influences the growth of the *B. typhosus*, the latter disappearing by the eighteenth day. Experiments similarly conducted upon the propagation of the *B. typhosus* in fresh water have yielded similar results. Thus Kraus showed that a very rapid decline of the bacillus, and a very rapid increase of the ordinary sea-water bacteria, took place when the water was incubated. Frankland and Ward showed that the bacillus disappeared at the end of thirty-four days in unsterilized Thames water, and that there was no multiplication in potable water.

How far all these laboratory experiments may be taken as indicating what actually takes place in nature, it would be difficult to say. The bacillus probably does perish in a short time in the sea, just as it does in sea-water in the laboratory, but

* Proc. Roy. Soc., vol. LVI., p. 440.

we have no evidence before us to show how far this is so in the case of the mud upon which the oysters may be laid; here it is possible that the bacillus might be capable of a saprophytic existence. The observations of Loesener, Robertson, and others concerning the duration of vitality of the *B. typhosus* in artificially infected earth, would tend to support such a view.

Tables showing the Action of Sea-water upon the Growth of the B. typhosus.

EXPERIMENT I.

	No. of Bacilli
At time of mixing	29,250
After 21 hours	20,475
„ 45 „	9,945
„ 71 „	9,360
„ 95 „	5,850
„ 271 „	260
„ 340 „	11

EXPERIMENT II.

At time of mixing	1,300
After 21 hours	1,105
„ 45 „	780
„ 71 „	650
„ 95 „	325
„ 271 „	2
„ 340 „	0

EXPERIMENT III.

At time of mixing	22,750
After 5 hours	17,550
„ 23 „	11,700
„ 48 „	3,250
„ 72 „	3,260
„ 247 „	455
„ 316 „	325

EXPERIMENT IV.

At time of mixing	130
After 6 hours	41
„ 23 „	31
„ 48 „	38
„ 72 „	negative
„ 247 „	1
„ 316 „	0

EXPERIMENT V.

At time of mixing	31,200
After 172 hours	9,360
„ 244 „	325

EXPERIMENT VI.

At time of mixing	325
After 172 hours	2

EXPERIMENT VII.

At time of mixing	325
After 504 hours (water kept at 8° C.—10° C.)	79

EXPERIMENT VIII.

At time of mixing	325
After 504 hours	0

III.—THE INFECTION OF THE OYSTER WITH THE B. TYPHOSUS, AND ITS REMOVAL BY WASHING.

These experiments were undertaken to ascertain whether oysters offered a favourable nidus for the multiplication of typhoid bacilli.

Healthy oysters were placed in large glass vessels containing sea-water, to which was added surface scrapings from agar plates previously inoculated with the typhoid bacillus.

By means of a syringe the water was aërated once or twice daily.

The contents of the stomach were pipetted out in the manner that will be indicated further on, and agar plates made; at the same time the water in which the oyster was placed was likewise analysed.

Table showing number of Organisms present in Stomach after infecting water.

Oyster.	Inoculated.	Examined.	No. of *Colonies.* Agar.	Organisms present in Oysters.	Number present in the Sea-water.
1	August 25.	August 26.	1,700	Almost entirely typhoid.	Water in the same case 585,000 per c.c.
2	"	"	"	"	
3	"	August 27.	7,020	"	Water in the same case 468,000 per c.c.
4	"	August 28.	7,000	"	Water in same case 40,950 on agar, 5,200 on gelatine.
5	August 26.	August 29.	453	"	
6	August 28.	August 30.	195	"	Water in same case 2,047,500 per c.c.
7	"	September 4.	390	"	
8	August 31.	"	325	"	
9	"	September 10.	455	"	

The above table shows that the typhoid bacillus does not increase in the body or in the tissues of the oyster. The figures would rather indicate, comparing the large number of bacilli present in the water with those found in the alimentary tract, that the bacilli perish in the intestine. We showed their presence up to ten days, and Dr. Klein up to two or three weeks.

In the next series of experiments infected oysters were taken, the duplicates of which, as seen in the above table, contained comparatively large numbers of the *B. typhosus*. They were subjected to a running stream of pure clean sea-water. The result was definite and uniform; there was a great diminution or total disappearance of the *B. typhosus* in from one to seven days.

Oyster.	Inoculated.	Washed.	Examined.	No. of *Colonies.* Agar.	Kind of Organisms present.
1	August 25.	August 26.	August 30.	80	2 Colonies, *B. typhosus.*
2	„	August 28.	„	23	*B. typhosus* present.
3	August 26.	„	„	44	„
4	„	August 29.	„	40	„
5	August 27.	„	„	5	„
6	„	„	August 31.	700	Abundant *B. typhosus.*
7	August 28.	August 30.	„	55	*B. typhosus* present.
8	August 26.	August 28.	September 3.	4	? *B. typhosus.*
9	August 27.	August 29.	„	10	No *B. typhosus* found.
10	„	„	„	8	3 Colonies of *B. typhosus.*
11	August 28.	August 30.	September 4.	4	1 Colony of *B. typhosus.*
12	„	September 3.	„	200	Majority *B. typhosus.*
13	August 31.	?	„	4	
14	August 28.	September 3.	September 6.	65	No *B. typhosus*, but *Proteus.*
15	August 31.	„	„	5	? *B. typhosus.*
16	„	September 5.	„	70	One-half Colonies *B. typhosus.*
17	„	September 3.	September 10.	1	No *B. typhosus.*
18	„	September 5.	September 11.	2	? *B. typhosus.*

IV.—The Bacteria present in the Alimentary Canal of the Oyster and the Mussel and other Shellfish.

The method of analysis consisted in first cauterizing the mantle over the region of the stomach and then inserting a fine sterilized glass pipette; the pipette was moved about, and when sufficient of the contents of the stomach and the juices of the oyster had risen in the pipette, the latter was removed and its contents transferred to liquefied agar, ordinary gelatine, or sea-water gelatine, and plate cultivations made. In the first six cases examined (*see* Table, A—F) the oysters were especially fresh; in all the other cases the shellfish had been obtained hap-hazard from the various shops. In a very large proportion of cases (⅓ to ½) the *B. coli* was isolated. In very many instances this organism was present in overwhelming numbers in the Petri dish.

Table showing number of Colon-like Organisms isolated from 48 batches of Oysters.

Source of Oysters.	No. of Colonies.		Bacillus isolated giving following reactions.					
	Agar.	Salt-water Gelatine.	Fermentation.	Indol.	Coagulation.	KI. Gelatine.	Motility.	—
From Sea. A.	0	Not made.						B. coli.
From Sea. B.	5							Not looked for.
From Sea. C.	0							
From Sea. D.	0							
From Sea. E.	1							Decolorized.
From Sea. F.	6							
Shop 2	108							
	1,040		Active.	Marked.	Marked.	Marked.	—	
	390							
.. 4	455							
	2							
	2		Active.	Marked.	Marked.	Marked.	Very motile.	Decolorized.
	102							
	350							
„ 5	12							
	1,170							
	21							
	195							
„ 6	5							
	20							
„ 7	3							
	2							
„ 8	3							
	70		Active.	None.	Marked.	Marked.	Motile.	Decolorized.
„ 9	9		Active.	None.	Marked.	Marked.	—	Decolorized.
	5							
	65							
	260							
„ 10	195							
	520							
„ 11	65							
	70		Active.	Marked.	Marked.	Marked.	Motile.	Decolorized.
„ 12	650							
	260		Active.	None.	Marked.	Marked.	Motile.	Decolorized.
„ 13	150		Active.	Marked.	Marked.	Marked.	Motile.	Decolorized.
	195							
„ 14	6							
	2		None.	None.	Marked.	Marked.	Motile.	Decolorized.
„ 15	100							
	5		Active.	Marked.	Marked.	Marked.	Motile.	Decolorized.
„ 16	20							
	70		Active.	Marked.	Marked.	Marked.	Motile.	Decolorized.
„ 18	25	3,025	Active.	Marked.	Marked.	Marked.	Motile.	Decolorized.
„ 19	1	2,330						
„ 20	265	13,000						
„ 21	100	1,755	Active.	Marked.	Marked.	Marked.	Motile.	Decolorized.
„ 22	35	2,330	Active.	Marked.	Marked.	Marked.	Motile.	Decolorized.
„ 23	15	3,025	Active.	Marked.	Marked.	Marked.	Motile.	Decolorized.
„ 24	40	6,500						
„ 25	5	13,000						
„ 26	2	8,775						
„ 29	325	17,550	Active.	Marked.	Marked.	Marked.	Motile.	Decolorized.
„ 30	50	20,475						
„ 31	65	2,925	Active.	Marked.	Marked.	Marked.	Motile.	Decolorized.

In a further series of experiments we examined nineteen batches of Oysters, seventeen batches of Mussels, eighteen batches of Cockles, five batches of Periwinkles, and one batch of Whelks: these were all obtained from shops in various parts of Liverpool.

Table II., showing Colon-like Organisms isolated from 19 *batches of Oysters.*

Oyster.	Bacillus isolated giving following reactions.					
	Motility.	Fermentation.	Milk.	Indol.	Potato growth.	Serum Reaction.
32	Actively motile.	Marked.	Marked.	Marked.	Abundant.	Negative.
33	Sluggish.	o	Peptonized.	Marked.	Abundant.	Negative.
34	Actively motile.	Marked.	o	o	Small.	Negative.
35	Actively motile.	o	o	o	Visible.	Negative.
36	Motile.	Marked.	Marked.	Marked.	Abundant.	Negative.
37	o	o	o	o	o	o
38	o	o	o	o	o	o
39	Actively.	o	o	o	Abundant.	Negative, forms alkali in neutral litmus whey.
40	o	o	o	o	o	o
41						
42						
43						
44	Actively.	Marked.	o	o	Abundant.	Negative, forms alkali in neutral litmus whey.
45	o	o	o	o	o	o
46						
47						
48						
49						
50						

Table III., showing Colon-like Organisms isolated from 18 *batches of Mussels.*

Mussels.	Motility.	Fermentation.	Milk.	Indol.	Potato Growth.	Serum Reaction and Remarks.
1	o	o	o	o	o	o
2	o	o	o	o	o	o
3	Active.	Marked.	Marked.	o	Abundant.	Negative, alkalinizes whey.
4	o	o	o	o	o	o
5						
6						
7	Active.	o	o	o	Abundant.	Negative, neutral reaction in litmus whey.
8	o	o	o	o	o	o
9	o	o	Marked.	o	o	o
10	Active.	o	o	o	Abundant.	Negative, alkali in neutral whey.
11	Active.	Marked.	Marked.	o	Variable.	Negative, neutral reaction.
12	o	o	o	o	o	o
13						
14						
15						
16						
17						
18						

Table IV., showing Colon-like Bacilli isolated from 18 *batches of Cockles.*

Cockles.	Motility.	Fermentation.	Milk.	Indol.	Potato growth.	Remarks.
1	o	o	o	o	o	o
2						
3						
4		Marked.	Marked.			
5	o	o	o	o	o	o
6						
7	Non-motile.	o	o	o	Variable.	Slight acid reaction.
8	o	o	o	o	o	o
9						
10						
11						
12						
13						
14						
15						
16						
17						
18						

Result of examination of 1 *batch of Whelks.*

Whelks.	Motility.	Fermentation.	Milk.	Indol.	Potato growth.	Remarks.
1		Slight.	0	0	Variable.	Slight acid reaction in neutral litmus whey.

The 5 batches of periwinkles yielded no colon-like bacilli.

The second series of results tends to confirm those of the first. In some instances there was almost a pure culture of the colon bacillus, the plate cultivations giving a very characteristic odour. It will be seen from all the tables that the "colon-like" bacilli isolated differ in some of their reactions. There are numerous examples of typical colon-like bacilli, coagulating milk, forming indol, gas, and giving a decided acid reaction. But there are several cases in which the bacilli are active, do not coagulate milk nor form indol, with very slightly acid or sometimes alkaline reaction. In these cases, which by their reactions approached the typhoid-like type, the serum reaction was carefully applied, but in no case was a positive result obtained. This indifferent colon group is of considerable interest, for it is quite possible that it might be confused with true typhoid. The tables also show that the oyster is more frequently liable to the presence of "colon-like organisms" than the other species of common edible shellfish examined. It is difficult to say what may be the significance of the so frequent appearance of this group of organisms. Dr. Klein is of opinion that the presence of the *B. coli* in the oyster is strongly suggestive of fouling of the particular sample with material of excremental origin. We are not yet prepared to go so far as this until we have learnt more about the normal occurrence of this organism in the animal kingdom.

V.—The occurrence of "Bacillus enteritidis sporogenes" in Shellfish.

We have recently examined samples of shellfish (oysters, mussels, cockles, and periwinkles) from the Liverpool shops, and find amongst them the frequent occurrence of large spore-forming anaërobic bacilli with the characteristics of Klein's *B. enteritidis sporogenes*. The interest of this consists in the recent demonstration* by Dr. Klein, that this bacillus occurs in certain cases of diarrhœa, in sewage, in manure, &c., and that its presence in articles of diet may be taken as presumptive evidence of fæcal contamination. The Medical Officer, in his last report to the Local Government Board, sums up the present position when he says,† "He (Dr. Klein) has, he believes, ground for regarding this bacillus as primarily derived from excrement, and for considering its presence in water, earth, and the like, as a more trustworthy criterion of direct fæcal pollution than the presence merely of *B. coli*."

* Twenty-seventh Annual Report of the Local Government Board for 1897-98. Medical Officer's Supplement.
† Loc. cit., p. 20.

We have considered it therefore of importance to examine specially for this organism a number of samples of oysters and other shellfish, as shown in the following table. The methods we have employed are these:—The shellfish have been carefully opened, and the water and "juices" surrounding the animal have been taken up in a sterilized pipette. A freshly-prepared milk tube, sterilized by boiling for one hour, is kept at a temperature of 80° C., and then inoculated and kept at 80° C. for fifteen minutes. The inoculated tube is then placed in a pyrogallate of soda tube (Buchner's method), and in addition the air replaced by running in hydrogen; the tube is firmly stoppered, and then transferred to the incubator and kept at 37° C. Well-marked results were obtained in 24 hours.

One cubic centimetre of the whey from the above tubes was inoculated subcutaneously in the groin of guinea-pigs. The results were noted after 24 hours.

Shellfish.	Sample.	Action on Milk in absence of Oxygen.	Results of Inoculation in Animals.	Remarks on Microscopic Preparations.
Oysters.	1	Coagulation. No gas.	No effect.	Short spore-bearing bacilli present.
"	2	None.	—	
"	3	None.	—	
"	4	Coagulation. Gas formed.	Local necrosis.	
"	5	Slight coagulation. Slight gas formation.	Swelling.	
"	6	Marked coagulation. Abundant gas.	Swelling.	
"	7	None.	—	
"	8	None.	—	
"	9	None.	—	
"	10	None.	—	
"	11	None.	—	
Mussels.	1	Coagulation. Gas formation.	Swelling.	Large spore-bearing anaërobic bacilli present.
"	2	Marked coagulation. Gas formation.	Swelling.	Do.
"	3	Do. do.	Swelling.	
"	4	Do. do.	Swelling.	
"	5	Do. do.	Nothing.	
"	6	None.	—	
Cockles.	1	Marked coagulation. Gas formation.	Extensive necrosis and swelling.	Small bacilli present.
"	2	Do. do.	Do.	Large spore-bearing anaërobic bacilli present.
"	3	Do. do.	Do.	
"	4	Slight coagulation. No gas.	Slight local reaction.	
Periwinkle.*	1	Coagulation. Gas formation.	Slight swelling.	Large spore-bearing anaërobic bacilli present.

* Large *Littorina littorea* from the west coast of Scotland.

This Table indicates that, so far as regards anaërobic milk-coagulating bacilli, oysters as bought in the town shops are considerably better than the other shellfish. The inoculations, however, were not in any case followed by death within 24 hours; while simultaneous experiments, in which the anaërobic milk-coagulating, spore-bearing bacilli were isolated from sewage, gave fatal results in that time. We are thus not yet prepared to say whether the bacilli from the shellfish are distinct from *B. enteritidis*, or represent attenuated (less virulent) conditions of Klein's form.

It is evident from the result of these experiments, and a consideration of all the facts brought to light in recent years in regard to the bacteriology of shellfish and its influence on public health, that we must regard oysters, mussels, cockles, and the like as nutritious food matters which, from their nature and the circumstances of their cultivation and sale, are liable to become contaminated with organisms—pathogenic or otherwise—and their deleterious products.

Once this is recognised the practical applications are largely a matter of common sense. Shellfish must not be taken as food from grounds where there is any possibility of sewage contamination; after removal from the sea, while in transit, in store, or in market, they should be carefully protected from any possibility of insanitary environment; they should not be kept longer than is absolutely necessary in shops, cellars, &c., in towns, where, even if not running the risk of fresh contamination, they are under conditions favourable to the reduction of their vitality and the growth of their bacterial contents—the fresher they are from the sea the more healthy they are likely to be; finally, only absolutely fresh shellfish should be eaten uncooked, and those that are cooked must be *sufficiently cooked*, raised to boiling point and kept there for at least ten minutes.

XIV.—CONCLUSIONS.

1. Although our primary object was to study the oyster under unhealthy conditions, in order to elucidate its supposed connection with infective disease, we found it necessary to investigate in minute detail the histology of certain parts of the body—especially the gills and mantle-lobes, the alimentary canal and the liver. We give figures and descriptions of parts of these structures in both normal and abnormal conditions.

2. We have also worked out the distribution and probable function of a minute muscle, which we believe to be the modified representative of the protractor pedis muscle of some other Molluscs.

3. A diseased condition we found in some of our experimental oysters very soon brought us in contact with the vexed question of "greening" in oysters, and one of the first results we arrived at was that there are several distinct kinds of greenness in oysters. Some of these, such as the green Marennes oysters and those of some rivers on the Essex coast, are healthy; while others, such as some Falmouth oysters containing copper, and some American oysters re-bedded on our coast, and which have the pale green leucocytosis we described in a paper to the Royal Society, are not in a healthy state.

4. Some forms of greenness (*e.g.*, the leucocytosis) are certainly associated with the presence of a greatly increased amount of copper in the oyster; while other forms of greenness (*e.g.*, the Marennes) have no connection with copper, but depend upon the presence of a special pigment (marennin), which may possibly be associated with a certain amount of iron.

5. We see no reason to think that the iron in the latter case is taken in through the surface epithelium of the gills and palps, but regard it, like the rest of the iron in the body, as a product of ordinary digestion and absorption in the alimentary canal and liver.

6. We do not find that there is any excessive amount of iron in the green Marennes oyster compared with the colourless oyster, nor do the green parts (gills, palps, &c.) of the Marennes oyster contain, either absolutely or relatively to the colourless parts (mantle, &c.), more iron than colourless oysters. We therefore conclude that there is no connection between the green colour of the "Huîtres de Marennes" and the iron they may contain.

7. On the other hand, we do find by quantitative analysis that there is more copper in the green American oyster than in the colourless one; and more proportionately in the greener parts than in those that are less green. We therefore conclude that their green colour is due to copper. We also find a greater quantity of iron in these green American oysters than in the colourless; but this excess is, proportionately, considerably less than that of the copper.

8. In the Falmouth oysters, containing an excessive amount of copper, we find that much of the copper is certainly mechanically attached to the surface of the body, and is in a form insoluble in water, probably as a basic carbonate. In addition to this, however, the Falmouth oyster may contain a much larger amount of copper in its tissues than does the normal colourless oyster. In these Falmouth oysters the cause of the green colour may be the same as in the green American oyster.

9. By treating sections of diseased American oysters under the microscope with potassium ferrocyanide and various other reagents, we find that the copper reactions correspond in distribution with the green colouration; and we find, moreover, from these micro-chemical observations that the copper is situated in the blood-cells or leucocytes, which are greatly increased in number. This condition may be described as a green leucocytosis, in which copper in notable amount is stored up in the leucocytes.

10. We find that an aqueous solution of pure hæmatoxylin is an extremely delicate test for copper, just as Macallum found it to be for iron.

11. Experiments in feeding oysters with weak solutions of various copper and iron salts gave no definite results—certainly no clear evidence of any absorption of the metals accompanied by "greening."

12. Although we did not find the *Bacillus typhosus* in any oysters obtained from the sea or from the markets, yet in our experimental oysters inoculated with typhoid we were able to recover the organism from the body of the oyster up to the tenth day. We show that the typhoid bacillus does not increase in the body or in the tissues of the oyster, and our figures indicate that the bacilli perish in the intestine.

13. Our experiments showed that sea-water was inimical to the growth of the typhoid bacilli. Although their presence was demonstrated in one case on the 21st day after addition to the water, still there appeared to be no initial or subsequent multiplication of the bacilli.

14. In our experiments in washing infected oysters in a stream of clean sea-water the results were definite and uniform: there was a great diminution or total disappearance of the typhoid bacilli in from one to seven days.

15. The colon group of bacilli is frequently found in shellfish as sold in towns, and especially in the oyster; but we have no evidence that it occurs in Mollusca living in pure sea-water. The natural inference, that the presence of the colon bacillus invariably indicates sewage contamination, must, however, not be considered established without further investigation.

16. The colon group may be separated into two divisions—(1) those giving the typical reactions of the colon bacillus, and (2) those giving corresponding negative reactions, and so approaching the typhoid type; but in no case was an organism giving all the reactions of the *B. typhosus* isolated. It ought to be remembered, however, that our samples of oysters, although of various kinds and from different sources, were in no case, so far as we are aware, derived from a bed known to be contaminated or suspected of typhoid.

17. We have shown the frequent presence, in various shellfish, of anaërobic spore-bearing bacilli showing the characteristics of the *B. enteritidis sporogenes* recently described by Klein.

18. Consequently, as the result of our investigations and the consideration of much evidence, both from the Oyster Growers and the Public Health Officer's point of view, we beg to recommend—

(*a*) That the necessary steps should be taken to induce the oyster trade to remove any possible suspicion of sewage contamination from the beds and layings from which oysters are supplied to the market. This could obviously be effected in one of two ways: either (1) by restrictive legislation and the licensing of beds only after due inspection by the officials of a Government Department; or (2) by the formation of an Association amongst the Oyster Growers and Dealers themselves, which should provide for the due periodic examination of the grounds, stores, and stock, by independent, properly qualified inspectors. Scientific assistance and advice given by such independent inspectors would go far to improve the condition of the oyster-beds and layings, to reassure the public, and to elevate the oyster industry to the important position which it should occupy.

(*b*) Oysters imported from abroad (Holland, France, or America) should be consigned to a member of the suggested "Oyster Association," who should be compelled by the regulations to have his foreign oysters as carefully inspected and certified as those from his home layings. A large proportion of the imported oysters are, however, deposited in our waters for such a period before going to market, that the fact of their having originally come from abroad may be ignored. If this period of quarantine were imposed upon all foreign oysters, a great part of the difficulty as to inspection and certification would be removed.

(*c*) The grounds from which mussels, cockles, and periwinkles are gathered should be periodically examined by scientific inspectors in the same manner as the oyster-beds. The duty of providing for this inspection might well, we should suggest, be assumed by the various Sea Fisheries Committees around the coast.*

* Since the above was written, and published in a preliminary abstract of this memoir, we are glad to learn that (1) the "British Oyster Industries Association" has been formed, and (2) an Oyster Bill has been laid before Parliament. If the Association takes a sufficiently high view of its responsibilities and duties, the provisions of the Bill will become unnecessary.

EXPLANATION OF THE PLATES.

The figures have been drawn either by ourselves, or under our immediate direction and supervision in our laboratories. The colours are not diagrammatic, but represent accurately the natural conditions and the effects of differential staining or chemical reactions.

Most of the histological work has been done with the Zeiss $\frac{1}{12}$ oil immersion and the Apochromatic 1.5 mm. and 2 mm. objectives, with the compensating ocular 12. Some of the figures have been drawn as seen under other powers, as indicated in the explanations.

PLATE I.

ILLUSTRATING THE GILL.

Fig. 1.—Diagram of the Anatomy of the Oyster (*Ostrea virginica*, Gm.) from the left side, natural size. *A.* anterior ; *P.* posterior ; *D.* dorsal ; *V.* ventral ; *a.* anus ; *add.* and *add.'* the two parts of the adductor muscle ; *au.* auricles of heart ; *br.* the two pairs of gills ; *c.g.* cerebral ganglion ; *cr.s.* crystalline style in first portion of intestine ; *h.d.* one of the hepatic ducts opening into stomach ; *i.* intestine ; *i.l.* the narrow intestinal loop ; *l.* liver ; *lig.* ligament ; *m.* mouth ; *p.p.'* anterior and posterior labial palps ; *p.m.* end of reduced protractor pedis muscle ; *p.h.* pre-oral hood of mantle or cucullus ; *pall.* pallium or mantle ; *r.* rectum ; *sh.* shell ; *st.* stomach ; *u.* umbo of shell ; *u.g.a.* urino-genital aperture ; *v.* ventricle of heart, in pericardium ; *v.g.* visceral ganglion.

Fig. 2.—Transverse section of part of a single branchia, to show its two lamella, their closely folded condition, and the large skeletal bars (× 40).

Fig. 3.—Transverse section across two crests of a single branchia, to show the gill filaments, an interlamellar junction, and the arrangement of the water and blood spaces. (Leitz, obj. 7).

Fig. 4.—A few gill filaments from one lamella, seen in section, to show their histology. (Zeiss, obj. $\frac{1}{12}$).

Fig. 5.—A few similar gill filaments from a Dutch green oyster (Nieuport, Jan. 19, 1897), to show the distribution of the eosinophilous macroblasts. (Zeiss, obj. $\frac{1}{12}$).

Fig. 6.—Piece of living gill of pale green "Native" oyster from West Mersea (Feb. 25, 1896), to show the active cilia and the green macroblasts. (Swift, 1 inch obj.)

Fig. 7.—The green macroblasts on three adjacent gill filaments. (Swift, $\frac{1}{4}$ inch obj.)

Fig. 8.—Row of macroblasts seen in profile. (Swift, $\frac{1}{4}$ inch obj.)

Fig. 9.—Two adjacent gill filaments to show macroblasts (*m*) and granular leucocytes (*l*). (Zeiss, $\frac{1}{12}$ oil immersion).

Fig. 10.—A macroblast and two adjacent epithelial cells. (Zeiss, apochromatic.)

Fig 11.—Blood-cells from gill (Zeiss, apochromatic 1.5 mm.)

Fig. 12.—Piece of free edge of gill of American oyster (June 8, 1898) showing leucocytes aggregated in numbers.

Fig. 13.—Another place on edge of gill showing the leucocytes migrating out. (Swift, $\frac{1}{4}$ inch obj.)

PLATE II.

ILLUSTRATING THE REDUCED PROTRACTOR PEDIS MUSCLE, &c.

Fig. 1.—American oyster, with half of the left valve (*c.s.*) removed, to show the insertion of the reduced pedal muscle at *sp.*

Fig. 2.—American oyster, with well-marked "spot" (*sp.*) formed by pedal muscle.

Fig. 3.—Another specimen, with the edge of the spot strongly pigmented (× 2).

Fig. 4.—Section across the adductor impression of the shell (× 2). The thin black line is dark brown pigment.

Fig. 5.—One end of a similar section magnified: *a*, prismatic layer; *b*, opaque chalky layer; *c*, transparent columnar lining to adductor impression; *d*, brown pigment (Swift, obj. 1 inch).

Fig. 6.—Three sections across the impression formed on shell by insertion of pedal muscle. The smaller black marks are brown pigment (× 2).

Fig. 7.—One end of a similar section magnified. Lettering as in Fig. 5 (Swift, obj. 1 inch).

Fig. 8.—Epithelium on surface of mantle near "spot," for comparison with,

Fig. 9.—Modification of surface of mantle at "spot" (Leitz, obj. 7).

Fig. 10.—Section showing the fibres of the pedal muscle leaving the spot and running inwards and backwards (Leitz, obj. 3).

Fig. 11.—Section further back, showing the muscle fibres coming into close relation with the large blood sinus (*lac.*) and with the renal tubules.

Fig. 12.—Section still further back, showing the fibres cut nearly transversely, and now lying below the blood sinus and near the liver tubules.

Fig. 13.—Last section of the series, showing the distribution of the muscle fibres along the top of the anterior end of the external gill (all Leitz, obj. 3).

Fig. 14.—Diagram to show the action of the modified protractor pedis muscle (*pr.*) in widening the two ciliated food avenues (see *arrows*) leading to the mouth (*m.*).

PLATE III.

ILLUSTRATING THE LIVER, &c.

Fig. 1.—Oblique section of the visceral mass and gills of an oyster, to show relations of stomach (*st.*), liver (*l.*), pallial lobes (*pall.*), and the 4 branchiæ (*br.*), natural size.

Fig. 2.—Section of a visceral mass, to show a dark coloured liver, natural size.

Fig. 3.—Similar section showing a light yellow brown liver, natural size.

Fig. 4.—Low-power view of a section of visceral mass of a green Dutch oyster, to show stomach (*st.*), intestine (*i.i.*), liver ducts (*d.d.d.*), and cæca of liver (*c.c.c.*); (Swift, obj. 1 inch).

Fig. 5.—Section showing the opening of a liver duct into the stomach (Leitz, obj. 3).

Fig. 6.—Longitudinal section of a liver cæcum (Leitz, obj. 7).

Fig. 7.—Transverse section of a liver cæcum (Leitz, obj. 7).

Fig. 8.—Transverse section of a well-formed liver cæcum of a green Dutch oyster (Leitz, obj. 7).

Fig. 9.—Three liver cells from the last, showing green granules in the cells (Zeiss, apochr. 1·5 mm.).

Fig. 10.—Section of a group of healthy, well-formed liver cæca (Zeiss, $\frac{1}{12}$ obj.).

Fig. 11.—Section of a group of shrunken, degenerate liver cæca, from a sickly Fleetwood American oyster (Zeiss, $\frac{1}{12}$ obj.).

Fig. 12.—Hepatic cells (*h.c.*), basal cells or "Fermentzellen" (*b.c.*), connective tissue (*c.t.*), green leucocyte (*l.*), from liver cæcum of green oyster with green granules (*gn. gr.*) in the liver cells (Zeiss, apochr. 1·5 mm.).

PLATE III.—*continued.*

Fig. 13.—Section showing stomach wall, liver duct, and cæca (Swift, obj. ¼ inch).

Fig. 14.—Epithelium of stomach wall (Zeiss, obj. $\frac{1}{12}$).

Fig. 15.—Epithelium of a larger liver duct (Zeiss, obj. $\frac{1}{12}$).

Fig. 16.—Epithelium of one of the smallest branches of the liver duct (Zeiss, obj. $\frac{1}{12}$).

Fig. 17.—Section of stomach wall of a green oyster, showing green pyriform macroblasts (Zeiss, obj. $\frac{1}{12}$).

Fig. 18.—Section to show the migration of leucocytes through the stomach wall (Leitz, obj. 7).

Fig. 19.—Section of stomach of green Dutch oyster, to show large eosinophilous granular cells (*eos*), and also green granular leucocytes (*g.l.*) migrating through the stomach wall (Leitz, obj. 7).

PLATE IV.

ILLUSTRATING GREEN OYSTERS.

Fig. 1.—"Huître de Marennes," with the right valve removed, and the mantle lobe partly reflected, to show the dark blue-green colour of the gills, natural size.

Fig. 2.—A small part of the gill enlarged with a lens, to show the vertical gill filaments arranged in three crests and two troughs.

Fig. 3.—A series of adjacent filaments examined alive to show the arrangement of the large macroblasts, and also some amœboid cells on the surface (Leitz, obj. 7).

Fig. 4.—The macroblasts *in situ*, more highly magnified, from filaments fixed in formaline, and teased (Zeiss, $\frac{1}{12}$ oil im.).

Fig. 5.—Epithelium of the intestinal wall of Marennes oyster, to show the pyriform green granular cells (Zeiss, $\frac{1}{12}$ oil im.).

Fig. 6.—Large Dutch oyster, showing the pale green gills.

Fig. 7.—External surface of mantle of an oyster from Nieuport, to show large granular eosinophilous cells, and also green granules in the epithelium (Zeiss, obj. $\frac{1}{12}$).

Fig. 8.—Another section of same to show large green granules under the epidermis (Zeiss, obj. $\frac{1}{12}$).

Fig. 9.—"Native" from the Roach River, to show the pale green gills.

Fig. 10.—Section of intestine of green Nieuport oyster, unstained and examined fresh to show colour of epithelium and liver tubules (Swift, 1 inch).

Fig. 11.—American oyster, living in sea-water after removal of left valve, to show corrugation of mantle lobe, and spotting of mantle and adductor muscle with masses of green leucocytes.

PLATE V.

ILLUSTRATING THE HISTOLOGY.

Fig. 1.—Section of edge of the mantle of Fleetwood American oyster, in which there is the green leucocytosis; showing vacuolated and disorganised epithelium through which the large granular leucocytes have been migrating (Zeiss, $\frac{1}{12}$ oil im.).

Fig. 2.—Section showing external surface of mantle in Marennes oyster, showing large eosinophilous cells (Zeiss, apochr. 1.5 mm.).

Fig. 3.—Transverse section of part of gill filament in Marennes oyster, showing eosinophilous cells (Zeiss, apochr. 1.5 mm.).

Fig. 4.—Eosinophilous cells in the epithelium and in the sub-epithelial connective tissue of Nieuport oyster (Zeiss, apochr. 1.5 mm.).

PLATE V.—*continued.*

Fig. 5.—Section showing external surface of mantle of Nieuport oyster, showing eosinophilous cells passing outwards (Zeiss, apochr. 1.5 mm.).

Fig. 6.—Transverse section of pallial tentacle of Dutch oyster, showing eosinophilous cells (Leitz, obj. 7).

Fig. 7.—Section of internal surface of mantle of American oyster, showing eosinophilous cells (Zeiss apochr. 1.5 mm.).

Fig. 8.—Another section of the same, to show the hæmatoxylin reaction in the eosinophilous cells.

Fig. 9.—Large amœboid granular green leucocytes from the surface of gill and mantle in American oyster, with the green leucocytosis, bedded at Fleetwood. The four marked *b* were from the blood, the rest from the surface (Zeiss, apochr. 1.5 mm.).

Fig. 10.—Leucocytes lying between the liver tubules stained with pure hæmatoxylin (Zeiss, $\frac{1}{12}$).

Fig. 11.—Transverse section of a gill filament of green "native" from Roach River (Zeiss apochr. 1.5 mm.).

Fig. 12.—Section of the liver of an American oyster with the green leucocytosis, showing the hæmatoxylin reaction given by the leucocytes (× 60).

PLATE VI.

ILLUSTRATING THE GREEN LEUCOCYTOSIS IN AMERICAN OYSTERS.

Fig. 1.—A healthy well-grown "East River" American oyster.

Fig. 2.—The same with shell valve removed from right side, to show the nearly colourless mantle.

Fig. 3.—A similar American oyster (from Liverpool market, May 17, 1897) with left valve removed, to show the green leucocytosis. Engorged green blood channels are seen over the viscera and on the mantle edge, and the auricles of the heart are filled with green leucocytes.

Fig. 4.—Left side of another similar oyster (Liverpool market, May 18, 1897), where the pallial blood channels are very completely injected with green leucocytes.

Fig. 5.—Group of Portuguese oysters (*Ostrea angulata*), one of which is opened to show the green leucocytosis (July 1897).

Fig. 6.—An American oyster (*O. virginica*) re-bedded at Fleetwood, to show a completely green mantle (March 30, 1897).

Fig. 7.—Another, to show a completely green heart and a bright patch on mantle over viscera.

Fig. 8.—An English oyster (*O. edulis*) from Falmouth (July 3, 1897), to show the ventricle of the heart completely green, and the auricles brown.

Fig. 9.—Small Dutch oyster (*O. edulis*), to show green spots scattered over the mantle and adductor muscle (July 26, 1897).

PLATE VII.

ILLUSTRATING THE COPPER REACTIONS IN GREEN OYSTERS.

Fig. 1.—Part of the mantle of a green American oyster (May 27, 1897) showing the engorged blood channels and smaller blood spaces in the connective tissue, coloured red-brown after treatment with potassium ferrocyanide (Zeiss, 35 mm.).

Fig. 2.—Two ventricles of American oysters, one (*B*) green and one (*A*) colourless, shown fresh and also after treatment with potassium ferrocyanide.

Fig. 3.—Preparation of blood of green oyster, after treatment with potassium ferrocyanide, to show some of the leucocytes coloured red (Zeiss, obj. D).

PLATE VII.—*continued.*

Fig. 4.—Small piece of the mantle of green American oyster, after treatment with potassium ferrocyanide, and then left overnight in clove oil to clear, showing an engorged blood channel stained red (Swift, 1 inch obj.).

Fig. 5.—Section of the mantle edge of green American oyster, showing an engorged blood space, coloured after treatment with potassium ferrocyanide.

Figs. 6, 7, 8, 9.—Four similar sections of the mantle edge of a green American oyster, showing a large blood space engorged with green leucocytes (Zeiss, 35 mm.).

Fig. 6.—Unstained, showing the dull green tint.

Fig. 7.—After potassium ferrocyanide.

Fig. 8.—After ammonium sulphide.

Fig. 9.—After pure hæmatoxylin.

PLATE VIII.

ILLUSTRATING THE HISTO-CHEMICAL REACTIONS.

Fig. 1.—Leucocytes in the mantle of the American green oyster, unstained (Zeiss, apochr. 2 mm.).

Fig. 2.—Another part of the same, treated with pure hæmatoxylin (Zeiss, apochr. 2 mm.).

Fig. 3.—The same, treated with potassium ferrocyanide (Zeiss, apochr. 2 mm.).

Fig. 4.—The same, treated with ammonium sulphide (Zeiss, apochr. 2 mm.).

Fig. 5.—A blood space in the mantle filled with leucocytes, treated with potassium ferrocyanide (Swift, $\frac{1}{6}$ inch).

Fig. 6.—A similar blood space, treated with pure hæmatoxylin (Swift, $\frac{1}{6}$ inch).

GEORGE PHILIP AND SON, PRINTERS, LONDON AND LIVERPOOL.

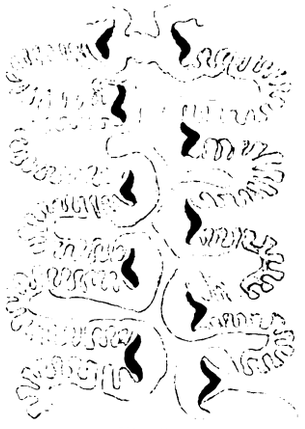


Fig. 2.

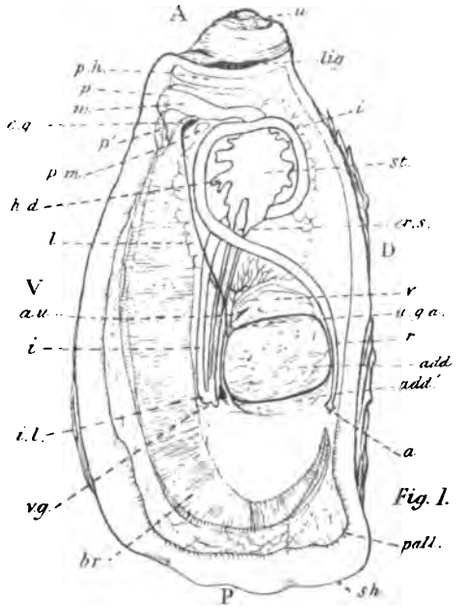


Fig. 1.

Fig. 4.

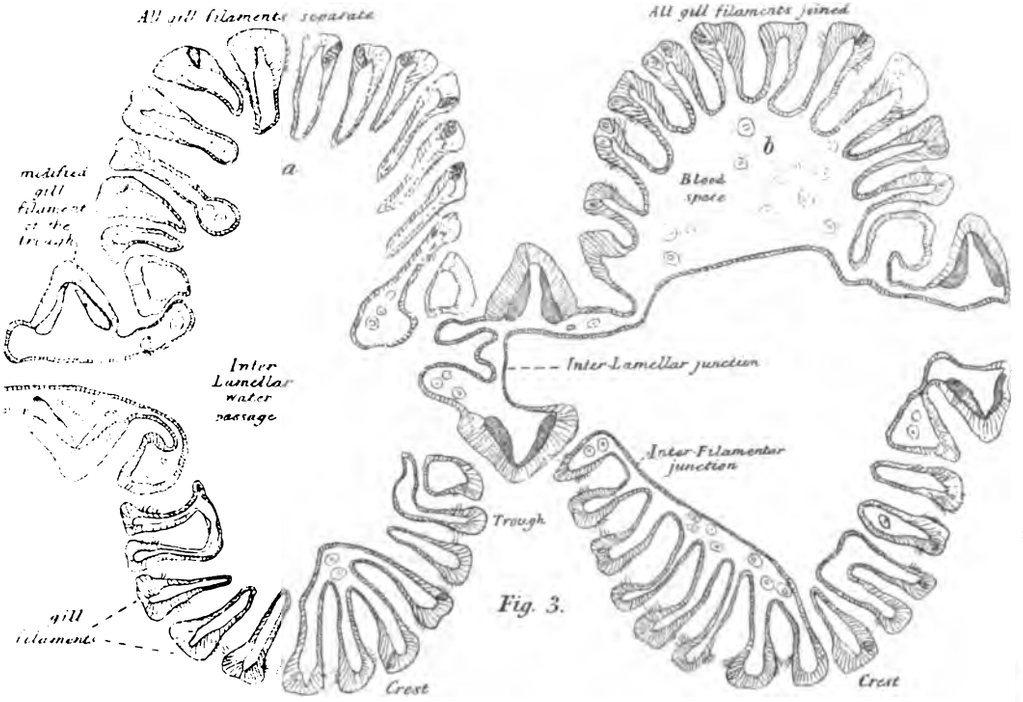


Fig. 3.

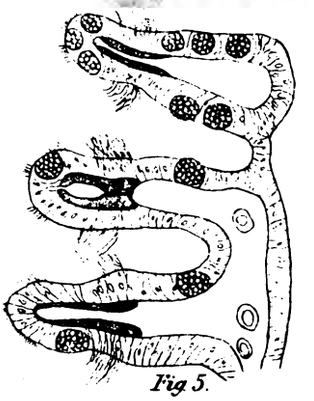


Fig. 5.

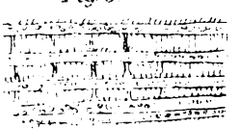


Fig 6

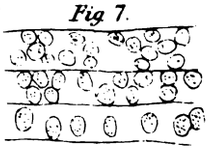


Fig. 7.

Fig. 8.

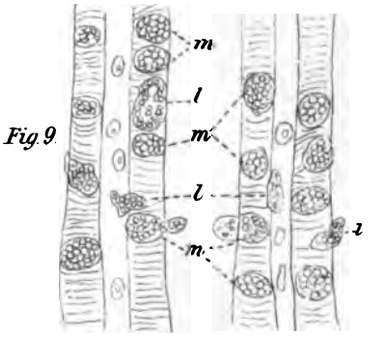


Fig. 9.

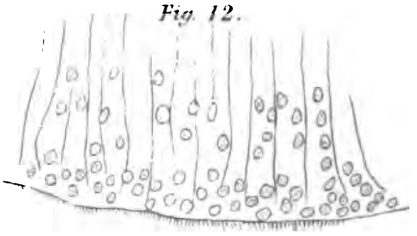


Fig. 12.

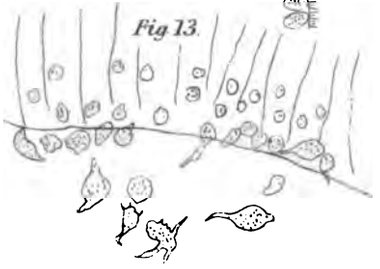


Fig 13.

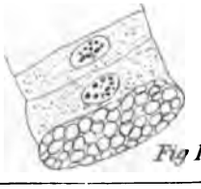


Fig 10.

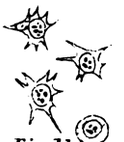


Fig 11.

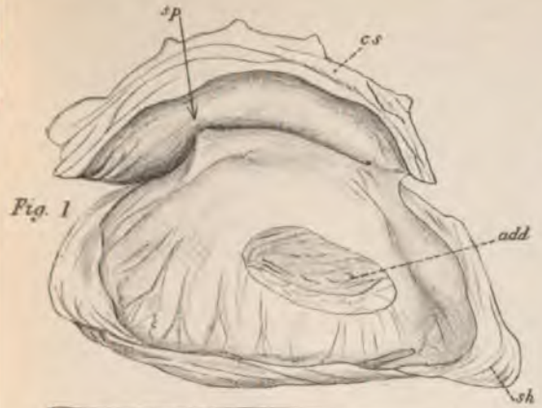


Fig. 1

Fig. 3.

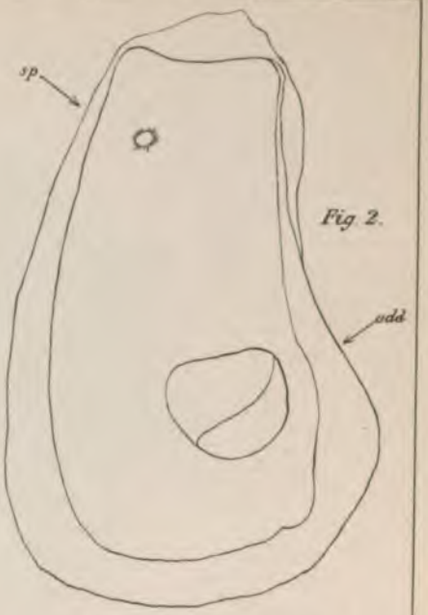


Fig. 2.

Fig. 4.

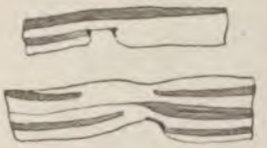


Fig. 6.

Fig. 5.

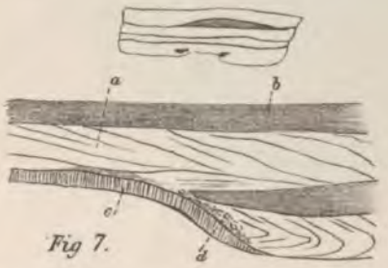


Fig. 7.

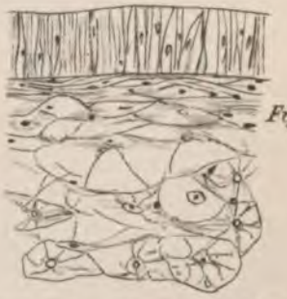


Fig. 8.

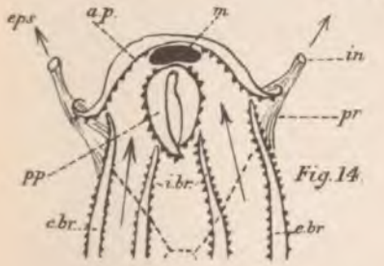


Fig. 14.

Fig. 10.

Fig. 9.

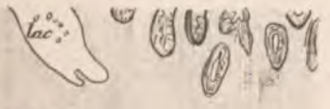


Fig. 13.

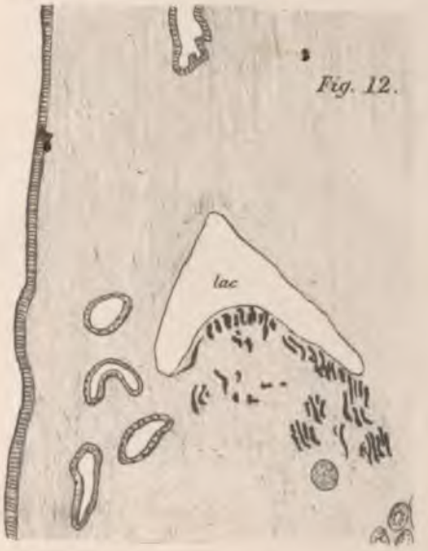


Fig. 12.

Fig. 11.

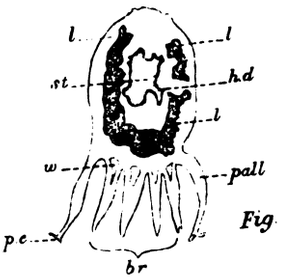


Fig. 1.

Fig. 2.

Fig. 3.

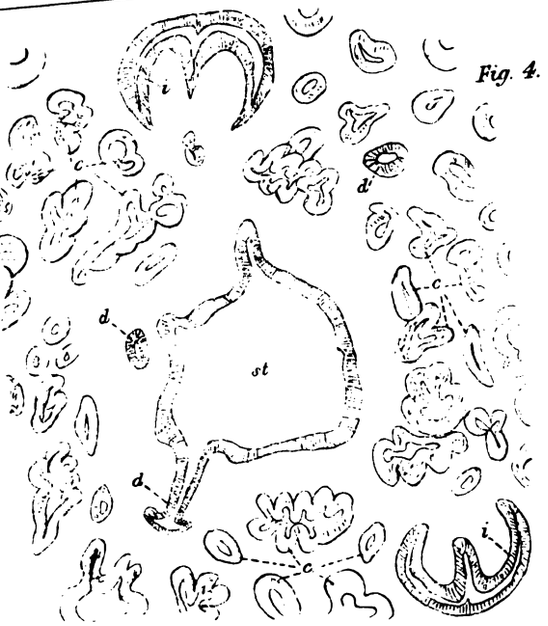


Fig. 4.

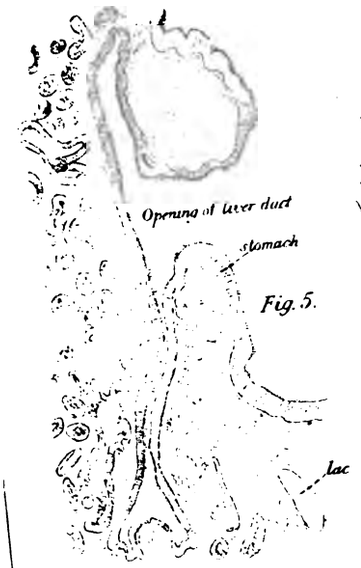


Fig. 5.

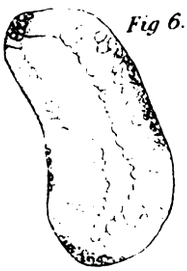


Fig 6.

Fig. 7.

Fig 8.

Fig. 9.

Fig 11.

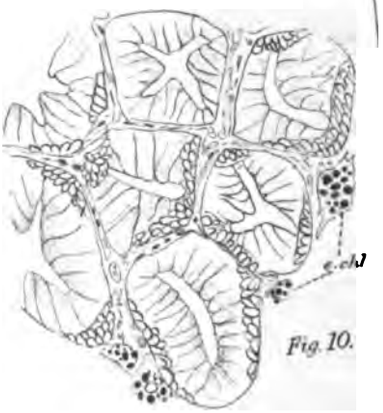


Fig. 10.

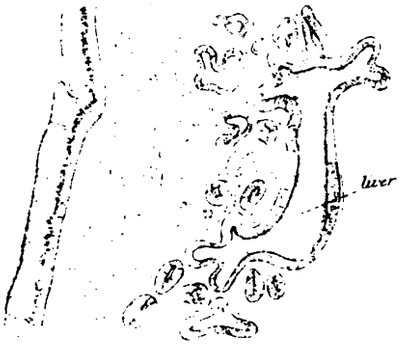


Fig. 13.

Fig. 16.

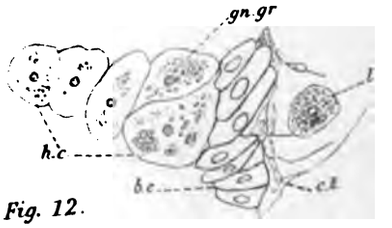


Fig. 12.

Fig. 15.

Fig. 14

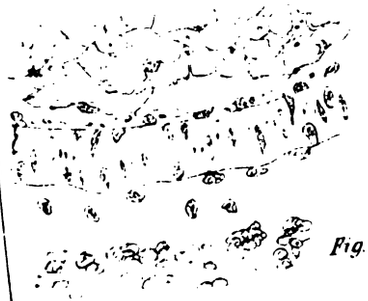


Fig. 18.

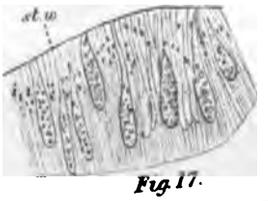


Fig. 17.

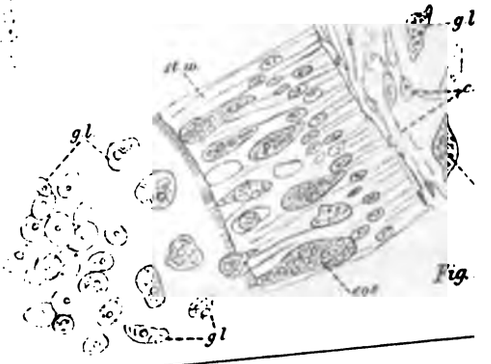


Fig.

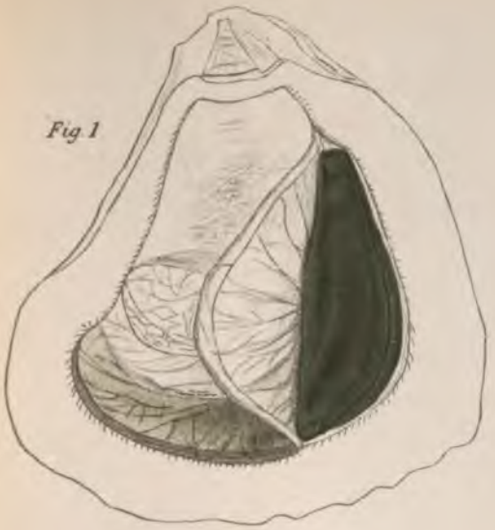


Fig. 1

Fig. 2.

Fig. 3.

Fig. 5.

Fig. 4.

Fig. 7.

Fig. 6.

Fig. 8.

Fig. 9.

Fig. 10.

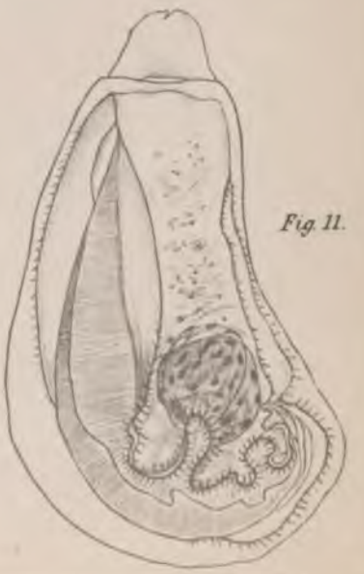


Fig. 11.

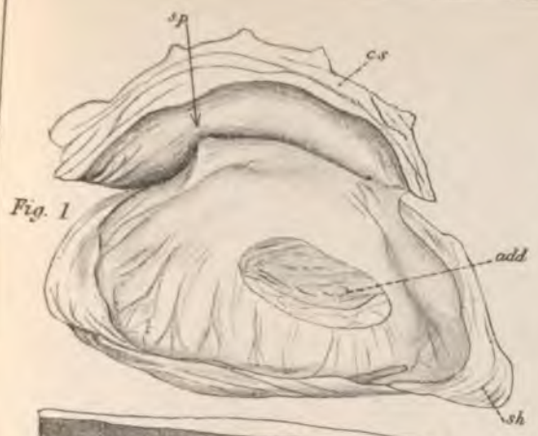


Fig. 1.

Fig. 3.

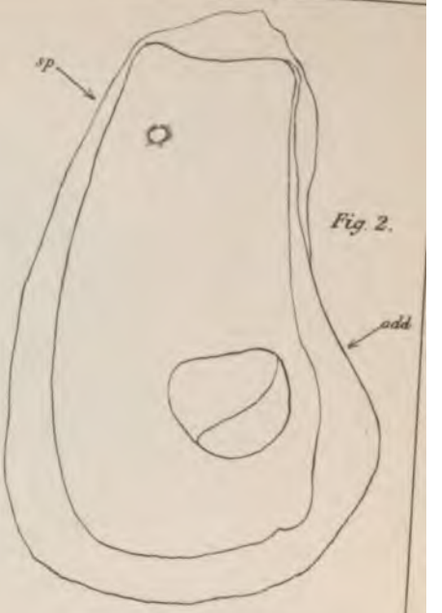


Fig. 2.

Fig. 4.

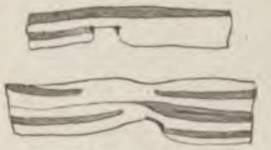


Fig. 6.

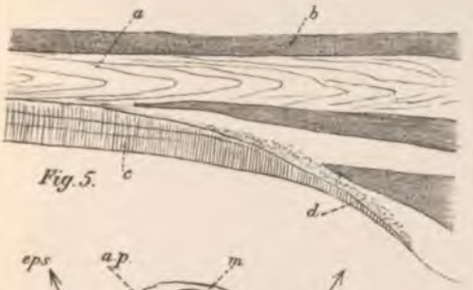


Fig. 5.

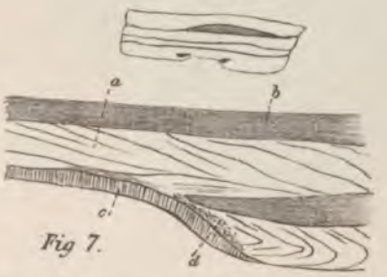


Fig. 7.

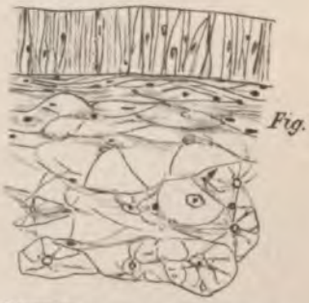


Fig. 8.

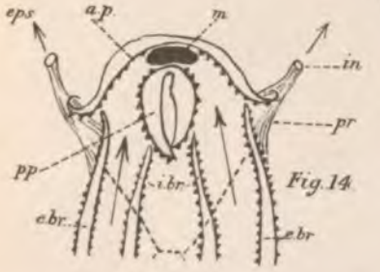


Fig. 14.

Fig. 10.

Fig. 9.

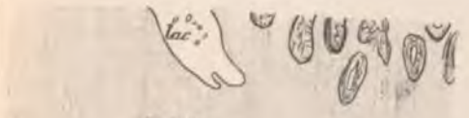


Fig. 13.

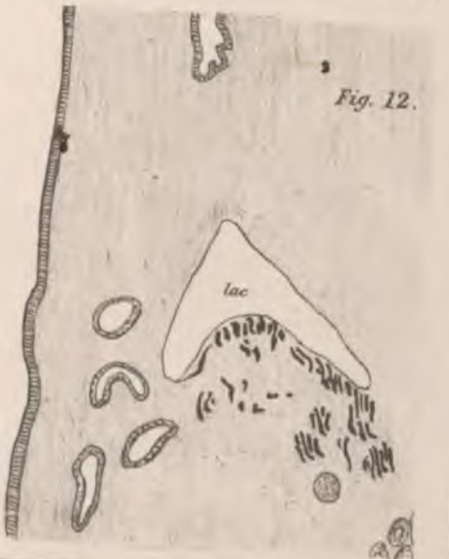


Fig. 12.

Fig. 11.

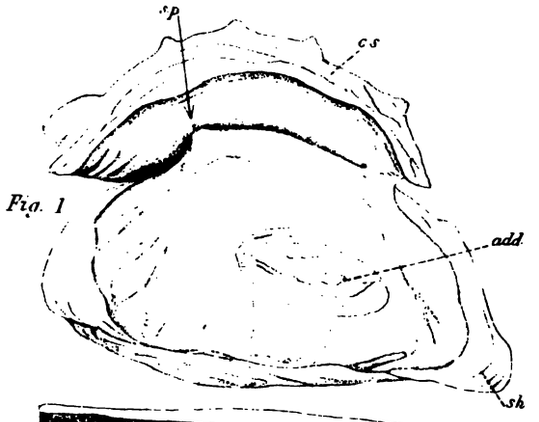


Fig. 1

Fig. 4.

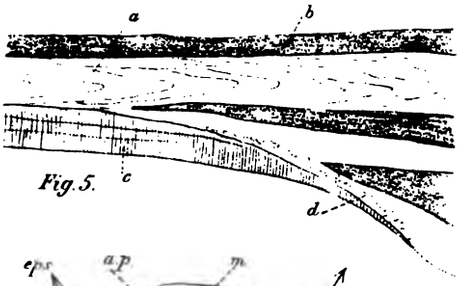


Fig. 5.

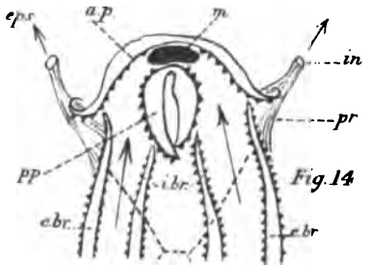


Fig. 14

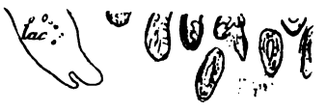


Fig. 13.

Fig. 3.

Fig. 6.

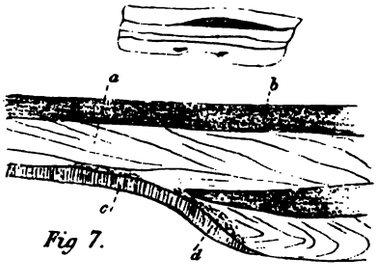


Fig 7.

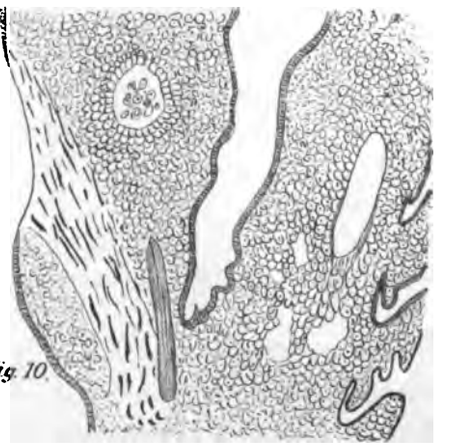


Fig. 10

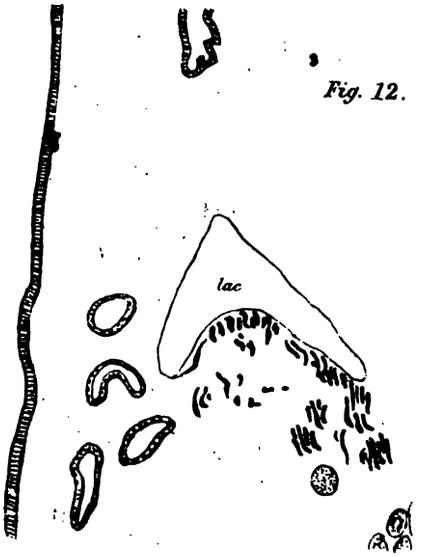


Fig. 12.

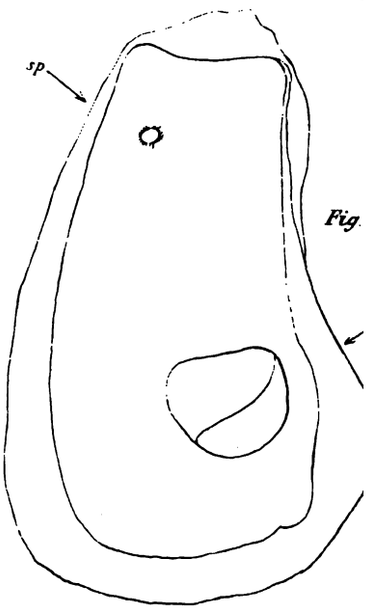
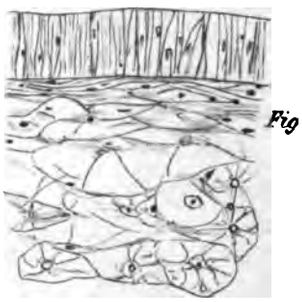


Fig.



Fig

Fig. 9

Fig. 11.

Fig. 1

Fig. 2.

Fig. 3.

Fig. 5.

Fig. 4.

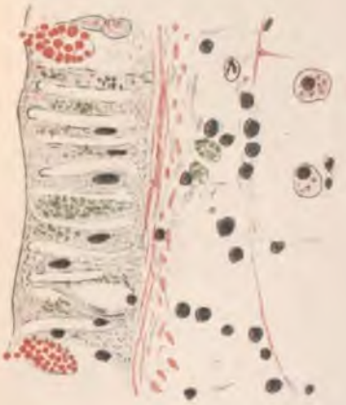


Fig. 7.

Fig. 6.

Fig. 8.

Fig. 9.

Fig. 10.

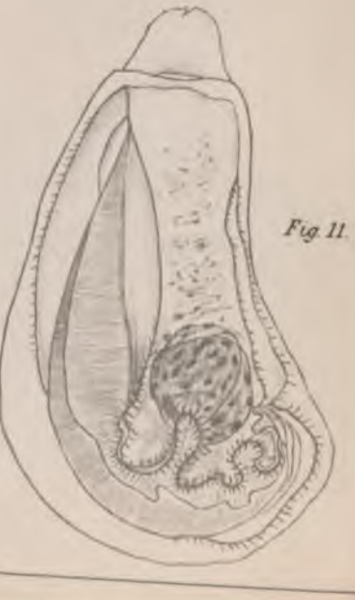


Fig. 11.

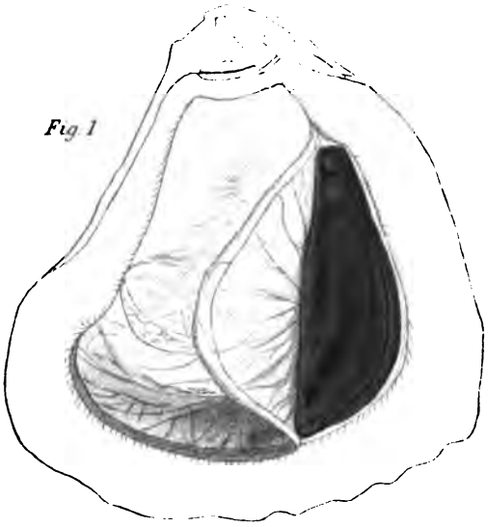


Fig. 1

Fig. 2.

Fig. 3.

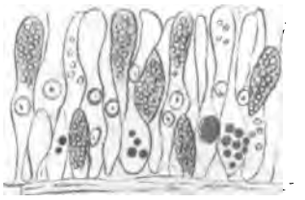


Fig. 5.

Fig. 4

Fig. 7.

Fig. 6.

Fig. 8.

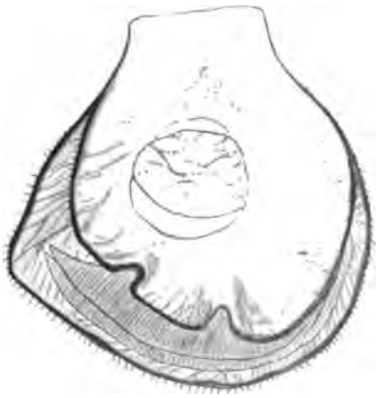


Fig. 9.

Fig. 10.

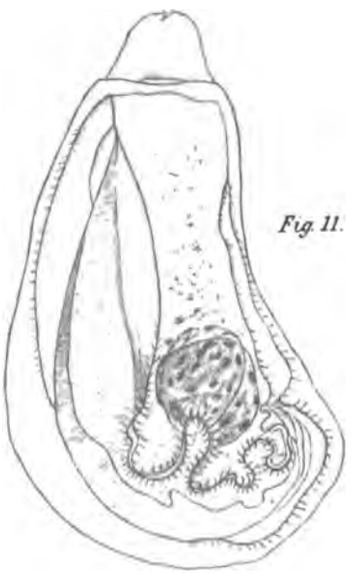
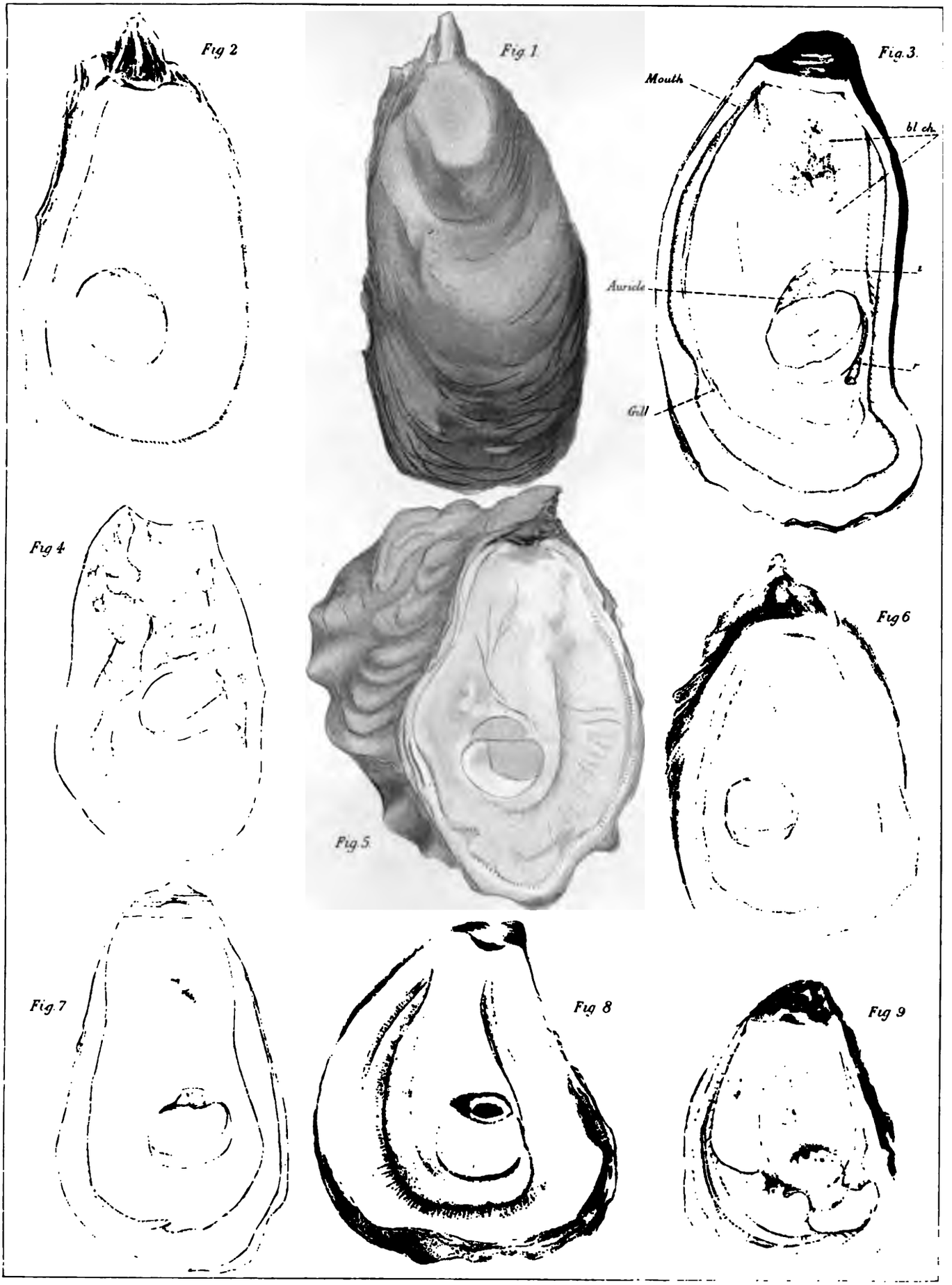


Fig. 11.



GREEN LEUCOCYTOSIS.

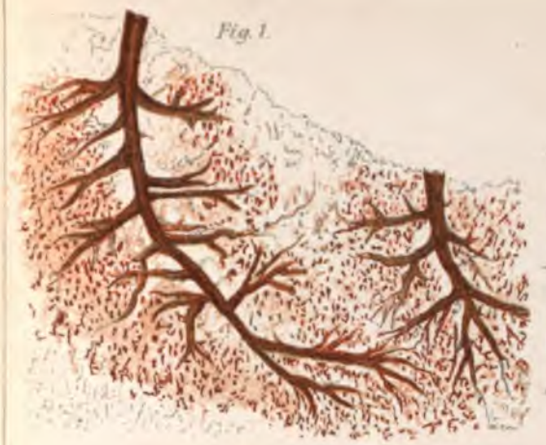
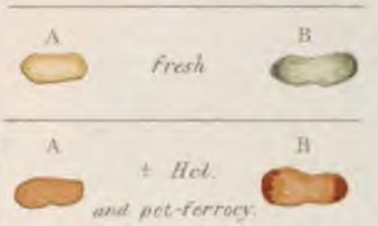


Fig. 1.

Fig. 2.



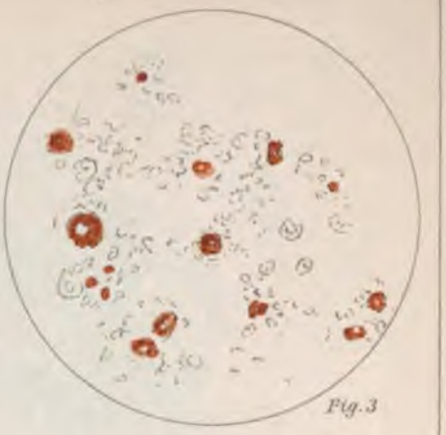


Fig. 3

Fig. 4.

Fig. 9.

Fig. 5

Fig. 6.

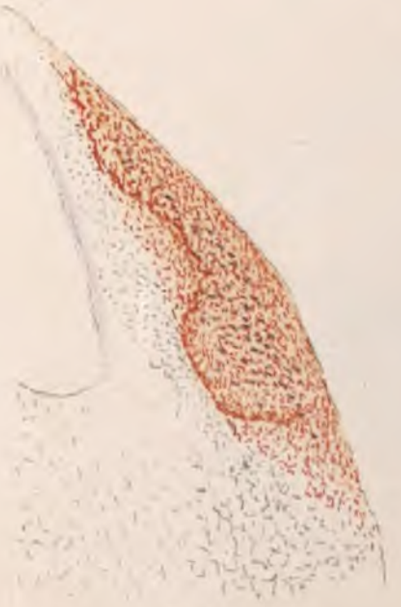


Fig. 7.

Fig. 8.

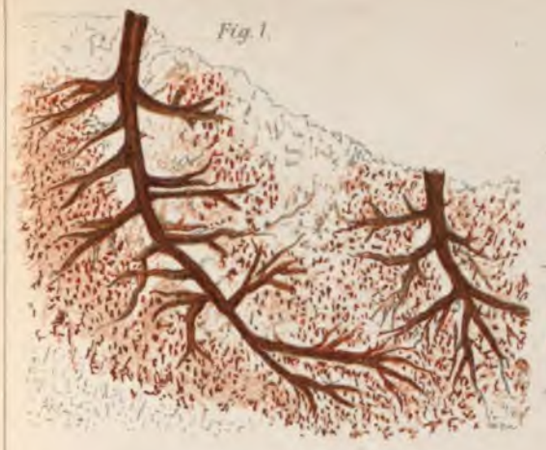


Fig. 1.

Fig 2.

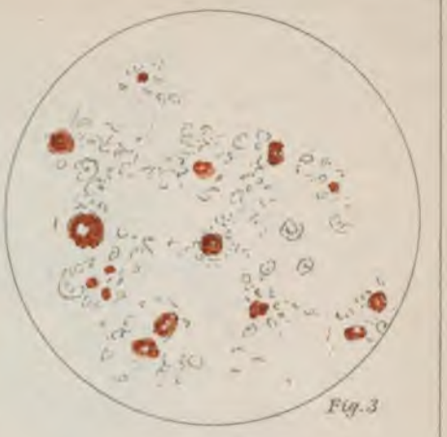


Fig. 3

Fig. 4.

Fig. 9.

Fig. 5

Fig. 6.

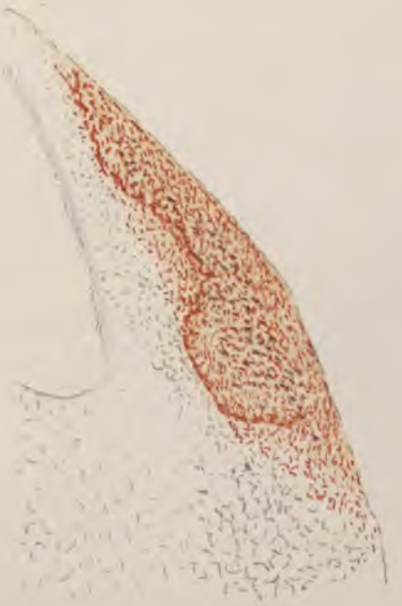


Fig. 7

Fig. 8.

REPORT

OF THE

MALARIA EXPEDITION

LONDON:
GEORGE PHILIP & SON 32 FLEET STREET

LIVERPOOL:
PHILIP SON & NEPHEW 45 TO 51 SOUTH CASTLE STREET

LIVERPOOL SCHOOL OF TROPICAL MEDICINE—MEMOIR II.

REPORT

OF THE

MALARIA EXPEDITION

OF THE

LIVERPOOL SCHOOL OF TROPICAL MEDICINE
AND MEDICAL PARASITOLOGY

BY

RONALD ROSS, D.P.H., M.R.C.S.
LECTURER IN TROPICAL MEDICINE UNIVERSITY COLLEGE LIVERPOOL

H. E. ANNETT, M.D., D.P.H.
DEMONSTRATOR IN TROPICAL PATHOLOGY

E. E. AUSTEN
ZOOLOGICAL DEPARTMENT BRITISH MUSEUM

WITH SUPPLEMENTARY REPORTS

BY MAJOR G. M. GILES, M.B., F.R.C.S., INDIAN MEDICAL SERVICE
AND R. FIELDING-OULD, M.A., M.B.

WITH ILLUSTRATIONS PLATES AND MAPS

Price 10s. 6d. net

AT THE UNIVERSITY PRESS OF LIVERPOOL 1900

PRINTED AT THE UNIVERSITY PRESS OF LIVERPOOL

No. 4. Feb., 1900. 1500 *Copies printed.*

TO THE

RIGHT HONOURABLE JOSEPH CHAMBERLAIN, M.P.

HER MAJESTY'S PRINCIPAL SECRETARY OF STATE
FOR THE COLONIES

WHO IN HIS OWN CITY HAS FOUNDED A UNIVERSITY

AND

DIRECTED RESEARCH TO THE ALLEVIATION OF TROPICAL MALADIES

THROUGHOUT VAST PROVINCES OF THE EMPIRE

THIS REPORT UPON THE PREVENTION OF MALARIAL FEVER

IN WEST AFRICA IS RESPECTFULLY DEDICATED

BY THE

LIVERPOOL SCHOOL OF TROPICAL MEDICINE

ISSUED BY THE COMMITTEE

OF THE

LIVERPOOL SCHOOL OF TROPICAL MEDICINE AND MEDICAL PARASITOLOGY.

COMMITTEE.

Alfred L. Jones, J.P., *Chairman.*
Wm. Adamson, *Vice-Chairman.*
R. T. Glazebrook,
Charles W. Jones, } Council of University College.
R. W. Boyce,
A. M. Paterson, } Senate of University College.
W. Alexander,
W. Carter, } Royal Southern Hospital.
J. O. Strafford, *Hon. Treasurer,*
E. Adam, } Chamber of Commerce.
J. W. Hughes,
Charles Booth, Jun., } Steamship Owners' Association.
Col. Goffey,
Jos. Chadwick, } Shipowners' Association.

A. H. Milne, *Secretary.*

Acknowledgments.

In publishing this Report the Committee wish to express their thanks, for assistance rendered in furtherance of the objects of the expedition, to the Right Honourable Joseph Chamberlain, Secretary of State for the Colonies; to Professor E. Ray Lankester, F.R.S., Director of the Natural History Department of the British Museum; to the British Medical Association; to H. E. Major Nathan, R.E., C.M.G., Acting Governor of Sierra Leone; to H. E. Sir Frederic Hodgson, K.C.M.G., Governor of the Gold Coast; to H. E. Sir William MacGregor, M.D., K.C.M.G., Governor of Lagos; to Colonel Burke, D.S.O., Commanding H. M. Forces in Sierra Leone; to Major Norris, D.S.O., and the Officers, 1st Batt. West India Regiment, Sierra Leone; to Drs. Strachan, Prout, Henderson, Renner, Berkeley, and Chalmers, of the West Coast; and to Major G. M. Giles, I.M.S., for kindly undertaking part of the work of Mr. Austen.

The present opportunity has been taken for recording some previous observations on the subject made in India by one of the members of the expedition.

ALFRED L. JONES,
Chairman.

CONTENTS

I. PRELIMINARY.

PAGE

1. Introduction - - - - - - - - - - 1
2. Objects of the expedition - - - - - - - - 2
3. History of the expedition - - - - - - - - - 2
4. Terminology employed - - - - - - - - 3

II. FREETOWN, AND FEVER STATISTICS.

5. Description of Freetown - - - - - - - - - 4
6. Statistics - - - - - - - - - - 5

III. HÆMAMŒBIDÆ IN FREETOWN.

7. The Hæmamœbidæ in intermediary hosts - - - - - 8
8. Methods for ascertaining definitive hosts - - - - - - - 9
9. Definitive hosts in Freetown - - - - - - - - 9
10. The Hæmamœbidæ in the definitive hosts - - - - - 13

IV. BIONOMICS OF THE CULICIDÆ IN FREETOWN.

11. Differences between *Culex* and *Anopheles* - - - - - - 15
12. Breeding-places of *Culex* - - - - - - - - 16
13. Breeding-places of *Anopheles* - - - - - - - - 16
14. Distribution of *Anopheles* in Freetown - - - - - - 18
15. Bionomics of *Anopheles* larvæ - - - - - - - - 19
16. Bionomics of adult *Anopheles* - - - - - - - - 20
17. Bionomical questions still requiring investigation - - - - - 24
18. Source of the Wilberforce *Anopheles* - - - - - - 25

V. DISCUSSIONS.

19. Questions regarding the parasites - - - - - - - - 26
20. Cause of occasional failures in direct cultivation - - - - - 29
21. Known facts about malaria explained - - - - - - - 30
22. Are other insects besides *Anopheles* hospitable to the human Hæmamœbidæ? - 31
23. Are other vertebrates besides man hospitable to the human Hæmamœbidæ? - - 32
24. Is the Life-history of the Hæmamœbidæ fully known? - - - - 33

VI. PREVENTION.

25. Measures indicated - - - - - - - - - 37
26. Precautions against the bites of gnats - - - - - - 38
27. Operations for reducing the numbers of *Anopheles* - - - - - 40
28. Under what conditions do operations agains *Anopheles* promise to be most successful 41
29. Recommendations - - - - - - - - - 43

VII. REMARKS.

30. Miscellaneous remarks - - - - - - - - - 45

ADDENDA.

1. Description of two species of *Anopheles*, by Major Giles, I.M.S. - - - 49
2. Observations at Freetown, Accra, and Lagos, by Dr. Fielding-Ould - - 51

REFERENCES.

PLATES AND MAPS.

REPORT OF THE MALARIA EXPEDITION

OF THE

LIVERPOOL SCHOOL OF TROPICAL MEDICINE AND MEDICAL PARASITOLOGY.

I. PRELIMINARY.

1.—Introduction.—The HÆMAMŒBIDÆ are a group of unicellular parasites of the red blood-corpuscle, found in man and in certain kinds of monkeys, bats, birds, and, perhaps, frogs. The group derives great importance from the fact that the human species are the cause of malarial fever.

We owe the discovery of these organisms to Laveran, Danilewsky, Koch, and Dionisi; and are indebted to Laveran, Golgi, Romanowsky, MacCallum, and others for our knowledge of their life-history and structure within the vertebrate hosts.

Following upon these discoveries, it remained to ascertain the life-history of the group outside these hosts; but all efforts to cultivate them or to find them in external nature were attended at first with failure. In 1883, however, King adduced a number of reasons in support of the view that gnats are connected with the propagation of malarial fever [1]. Next year Laveran expressed the opinion that the parasites discovered by him may undergo further development in gnats [2]. In 1894 Manson independently came to the same conclusion; chiefly on the ground that some forms of the parasites (gametocytes) emit certain motile filaments (microgametes) after the blood containing them is drawn from the host—a phenomenon which could be explained only by the theory that the parasites migrate into suctorial insects [3, 4]. Koch, Mendini, and Bignami have also adopted the gnat-theory of malaria on various grounds [12].

Working in India upon Manson's induction, one of us, after numerous negative experiments with several species of gnats of the genus *Culex*, succeeded in cultivating one of the human Hæmamœbidæ in two species of gnats of the genus *Anopheles* (dappled-winged mosquitoes) in 1897 [5]; and next year completed our knowledge of at least one cycle of the life-history of this group of parasites by following the development of *Hæmamœba relicta* of birds in gnats of the *Culex pipiens* type [6], and by communicating the infection from diseased to healthy birds by the bite of these insects [7]. This work was confirmed by Daniels in 1898-99 [8].

In 1897 MacCallum had already supplied an important fact in the life-history of the group by demonstrating the sexual nature of certain forms (gametocytes) of the organisms [9].

At the end of 1898, Koch and Grassi, Bignami and Bastianelli, almost simultaneously confirmed these observations. It was found that in Italy, as in India, the human species develop in gnats of the genus *Anopheles*; and the Italian observers finally succeeded in infecting healthy human beings by the bite of previously infected insects of this kind [10, 11].

The results of these investigations have been accepted by Ray Lankester, Laveran, Manson, Metchnikoff, and others; and a full history and bibliography of the subject has been published by Nuttall [12].

To sum up; although some points of importance still require investigation, it has been shown that certain species of gnats are definitive hosts of the Hæmamœbidæ; that the parasites are conveyed from diseased to healthy vertebrates (intermediary hosts) by these insects; and that the definitive hosts of the human parasites are certain species of *Anopheles* [*Section V.*].

2. Objects of the Expedition.—As soon as these facts were ascertained it became necessary to enquire whether the discovery could not be turned to practical account for the better prevention of malarial fever. The question was theoretically examined at length by one of us in February [13], and again in May, 1899 [14]. From an early stage in his investigations in India he had noted that, so far as his studies then went, gnats of the genus *Anopheles* sometimes appeared to breed only in comparatively small and isolated pools of water—a fact which suggested that in some localities it may be possible to exterminate them without great difficulty. Unfortunately his observations had not been exhaustive enough to enable him to speak with confidence on the point; while other students of the subject had not given it sufficient attention.

Hence the Liverpool School of Tropical Diseases undertook to open a special investigation of the question; and despatched an expedition consisting of ourselves to the West Coast of Africa for the purpose. Our objects were as follows:—

(1.) To find one or more species of insects hospitable to the human Hæmamœbidæ on the West Coast of Africa.

(2.) To study the bionomics of these insects with a view to suggesting better modes of prevention of malarial fever than those hitherto known to us.

It was not proposed that the expedition should remain in Africa for more than a few weeks; and it will therefore be understood that, owing to the short time at our disposal, we were obliged to limit our investigations strictly to the matter in hand.

3. History of the Expedition.—Sierra Leone was selected for the field of the first operations.

The expedition primarily consisted of (1) Major R. Ross, Lecturer in Tropical Medicine, University College, Liverpool; (2) Dr. H. E. Annett, Demonstrator in Tropical Pathology, Liverpool School of Tropical Diseases; and (3) E. E. Austen, Esq., Zoological Department, British Museum.

We arrived in Freetown, Sierra Leone, on the 10th August, 1899; and speedily succeeded in ascertaining that two species of gnats of the genus *Anopheles* found in Freetown are hospitable to the human Hæmamœbidæ [*Section III.*]. The rest of our time was spent chiefly in a study of the bionomics of these insects [*Section IV.*]; and we left Freetown for England on the 27th September, after seven weeks' stay in the colony.

Our observations constitute the body of this report; but before our departure from Sierra Leone we were joined by Dr. Fielding-Ould, who was despatched by the Committee of the School to continue our labours after our return. After we left, Dr. Fielding-Ould proceeded to Accra and Lagos; and his observations are given separately in the Addenda to this report.

We were also joined at Freetown by Dr. G. Van Neck from Belgium. Dr. Van Neck was unfortunately taken seriously ill shortly after our departure, and did not return until December.

4. Terminology Employed.—As regards the Hæmamœbidæ, we adopt the morphological terminology and taxonomic nomenclature already used by one of us in consultation with Professor Herdman, F.R.S. [15]. These will be found in the Discussions [*paragraph 19*]; where we consider the terminology further, and also give a summary of the life-history and classification of the parasites.

As there is no difference between gnats and mosquitoes—the latter term being merely one which is popularly employed for gnats in warm countries—and as this confusion tends to maintain erroneous notions regarding these insects, we propose to abandon the word *mosquito* and use in preference the old English equivalent *gnat*.

The terms *malaria* and *malarial fever* are unsound, while *paludism* is inexact. We may therefore venture to suggest the appellations *hæmamœbiasis* or *gnat-fever* for the disease which is the subject of this report. They will at least serve to popularise more correct notions of the malady than are implied in the word *malaria*.

II. FREETOWN, AND FEVER STATISTICS.

5. Description of Freetown.—Freetown, the Capital of the British Colony of Sierra Leone, is situated approximately in Lat. 8° 30′ N., and Long. 13° 15′ W., on the northern shore of a mountainous promontory. Close behind the town there is a semicircle of wooded hills rising to a height of 3,000 feet above sea-level ; and the town itself lies mostly on areas of level ground between them and the sea. The principal houses are built near the wharfs ; but from here the suburbs reach backward between the spurs of the hills. Few houses of the town proper exist on the slopes of the hills ; but numerous separate villages are found in the higher valleys above.

The soil of Freetown is derived from a red laterite rock ; which lies at little depth, and is exposed in many level parts of the town, as well as in water-courses, etc.

The rainy season lasts from May until October. The average annual rainfall is 163 inches, the average humidity 75.0, and the average temperature 80.0° F. [*paragraph 6*].

1. Freetown from Mount Aureole, looking westward. Tower Hill in the middle distance, and Wilberforce Hill in the distance.

The city contained 30,033 inhabitants at the last census (1891). The population consists mostly of natives (Christians, Mohammedans, Pagans), but there is also a colony of Europeans.

2. Street in Freetown, showing ditch at the side.

The streets are open spaces between the houses, generally fringed with grass, unpaved, and undrained, except by roadside ditches ; but gutters exist in the best part of the town, and occasionally elsewhere. Most of the streets are sloped, but a few are almost level.

The houses are built mostly of wood, or consist of a superstructure of wood on a masonry foundation. They are often two stories high, and are generally divided from each other by small open yards and gardens, in the manner of *villas*. As a rule the rooms are very small for tropical habitations.

There is an excellent water-supply brought from the adjacent hills in pipes.

Surface and storm-water drainage is carried mostly in the roadside ditches, but mountain torrents on their way to the sea occasionally intersect the level areas on which the town is built.

There is no sewage drainage, nor removal system for night-soil. Deep pits, or rather wells, covered by a privy and seat, are employed. It is stated that the old pits are sometimes filled up and new ones dug; but so far as we could judge, dry earth is seldom used, except by the Europeans and troops, and the system adopted seems to be a natural kind of *septic-tank* system.

Wells for drinking-water are not generally in use.

Horses and wheeled traffic are rare. Those who do not walk are carried by native bearers in "hammocks."

The troops, which belong mostly to the West India and West African regiments, are housed much better than the civil population. Their barracks are well-built wooden structures, placed on the summits of four hills; namely, on Tower Hill (400 feet), in the middle of the town; on Mount Aureole (800 feet) and Kortright Hill (1,100 feet), to the east of the town; and on Wilberforce Hill (600 feet), some distance to the west of the town [*photograph* 1 *and Map I.*].

Civil patients are treated in the large Colonial Hospital, the Incurable Hospitals, Lunatic Asylum, etc. Military sick have their own hospitals attached to the several barracks mentioned above.

Mangrove swamps occur in some of the bays and creeks of the shore.

Collections of water suitable for the larvæ of gnats abounded in the town when we were there (rainy season). Such collections could be divided into two classes; namely, (1) collections in vessels; and (2) collections on the ground. The former occurred in tubs, pots, broken bottles, etc., in the vicinity of almost all the houses; the latter chiefly in the roadside ditches and holes in rock, and, almost exclusively, on areas of level ground.

6. Statistics.—It is usually impossible to obtain from statistics any exact information regarding the actual number of infections of hæmamœbiasis occurring within a given place and period. The reasons for this are—(1) that the vast majority of attacks are not recorded; (2) that a large number of attacks which are recorded must be mere relapses, due to infections acquired perhaps long previously and in other localities; and (3) that the microscope is not generally used for exact diagnosis. Similarly the mortality returns are apt to be vitiated, because (1) the infection has often been acquired elsewhere; (2) Europeans are frequently invalided when seriously ill; and (3) deaths of cachectics are often ascribed to immediate causes, such as pneumonia and dysentery.

Military returns, though they are sometimes thought to be more trustworthy, are open to the same objections. Many soldiers do not come to hospital at all for slight attacks of fever; while others are frequently only "detained" in hospital for brief treatment, without being "admitted"—that is, entered in the returns. Thus we were informed of an instance in which only one quarter of the cases of fever which came to hospital had been "admitted" and recorded.

The *absolute* figures are therefore not trustworthy; but, by supposing the ratio of error to be always constant, we may derive some useful *comparative* information regarding the amount of fever prevalent in different places, and at different seasons.

We will content ourselves by giving a table extracted from the work of Major L. M. Wilson, R.A.M.C. [16]. The statistics are based on admissions to hospital among the troops in Sierra Leone during seven years (1892-98), and on the meteorological returns of the same period.

Average monthly Admission rates among the troops, and average monthly Rainfall, Humidity, and Temperature in Sierra Leone during seven years (1892-98).

Month.	Average admission rate per 1000.		Average Rainfall in inches.	Average Humidity.	Average Temperature in shade.
	Non-European.	European.			
January	52	93	.80	70.2	80.8
February	47	99	.86	68.2	81.0
March	63	59	1.24	67.7	81.7
April	86	106	4.36	67.3	81.6
May	99	225	14.08	75.3	80.3
June	128	347	18.62	77.1	79.2
July	171	413	33.34	83.0	78.3
August	116	281	38.96	84.0	77.7
September	91	173	29.85	82.2	78.8
October	67	121	14.63	79.1	79.4
November	72	125	5.41	75.0	80.5
December	64	92	1.26	71.9	80.7
Annual ...	1056	2134	163.41	75.0	80.0

Judging from these figures Wilson remarks, "The largest number of cases occur in the middle of the rainy season." Though many of the cases recorded in the rains may be relapses due to wettings, chills, etc., this can scarcely account for the quadruplication of the admissions shown in the table; and it would therefore appear that the *period of fresh infections* sets in (or at least increases) with the rains, and ceases (or at least diminishes) with them.

Wilson also thinks that "The white troops suffer more in proportion than the black." He gives the annual average death-rate of the white troops from malarial fever at 42.9 *per mille*, as against 5.9 *per mille* for the black troops. Similarly, he thinks that "Each battalion of the West India Regiment suffers exceptionally from fever the first year of its service on the coast." Such differences point rather to variations in *immunity*, than to variations in *frequency of infection* in the groups concerned.

It is hardly necessary to compare these statistics of Sierra Leone with similar figures for many other stations in the empire; but it will be instructive to give some ratios taken from the Annual Report of the Sanitary Commissioner with the Government of India for 1897.

For the whole European Army of India during that year the admissions *per mille* of strength for malarial fever were 420.1; and the deaths *per mille* were 1.0—far below the

figures for Sierra Leone. But in the most malarious stations the admissions *per mille* reached 1500; and the deaths, 6.0, excluding "remittent fever."

For the whole Native Army of India the annual admissions *per mille* were 362.8; and the deaths *per mille*, 1.7. But in the most malarious stations the admissions reached 2230, and the deaths, 6.8 *per mille*.*

It is obvious that Sierra Leone is much more malarious than India taken as a whole is; though perhaps not much more so than some places in India.

A study of the Indian statistics shows, moreover, that the admissions for the disease in that country have, roughly, the same dependence on rainfall as they have in Sierra Leone—that is, of course, not on the total amount of rainfall, but on its seasonal variations. Indeed, some of the most malarious military stations in India (Peshawur, Delhi, Lahore, etc.) have a small total rainfall.

* As a rule, natives in India suffer more than Europeans do—at least to judge by statistics.

III. HÆMAMŒBIDÆ IN FREETOWN.

7. The Hæmamœbidæ in Intermediary Hosts.—Duggan studied the human parasites of Sierra Leone some years ago [17]. He found only *Hæmomenas præcox* (with one exception—a case of *Hæmamœba vivax*). *Hæmomenas malariæ* was not observed by him. He says "Crescents were found in varying numbers in most of the cases." His results are based on the study of "about 400 cases."

Our own examinations of the blood of patients were made solely with a view to obtaining a basis for further researches as to the definitive hosts of the parasites; and it will be understood that we contemplated no morphological or pathological studies. Search was confined almost entirely to soldiers, few cases of fever among the civil population being procurable by us for study. The barracks at Wilberforce and Tower Hill afforded excellent fields for investigation. It should be noted that the men in these barracks belonged to West India regiments—that is, were natives of the West Indies who had been serving in Sierra Leone only for a short period.

The troops at Wilberforce had arrived there some months previously from a very malarious part of the colony, and continued to suffer severely from fever. They numbered about 320 men. We were informed that out of these no less than 195 men had come to hospital for fever in one month (July); although, as most of the men were not ill enough for admission, the "admission" rate was much lower. We examined the blood of a large number of the men, both in hospital and out of it, and estimated that the parasites were to be found in about a quarter of them taken at random. The species found were all of those now recognised in most parts of the world. *H. malariæ* was the commonest; but cases of *H. vivax* and *H. præcox* also abounded, being about equal in number. Cases of double infection were frequently observed. The prevalence of the disease was at its height at the time of our arrival, although quinine had been and was being sedulously given both for treatment and prophylaxis; and it was evident that such a number of cases could not be attributed alone to relapses, but that fresh infections were occurring at the time.

At Tower Hill there was much less fever. We attempted no estimate of the number of men in whom parasites could be found. Parasites could, however, be easily detected in the hospital cases. They belonged almost exclusively to *H. præcox*; though two or three cases of *H. malariæ* and *H. vivax* were observed.

There were very few cases among the troops on Mount Aureole and Kortright Hill. Of three cases examined in the hospital on Mount Aureole, one contained *H. malariæ*; another the melanin of *H. malariæ*; while the third was negative on a cursory search.

The morphology of these Hæmamœbidæ presented no differences from the accepted standards. One of us has been inclined to admit two varieties of *H. vivax*, one with fine light brown pigment, and one with fine black pigment. Both were observed in Freetown ; but the latter only in one case.

We were much struck by the absence or paucity of crescents (gametocytes) in cases of *H. præcox*. This fact will be discussed later [*paragraph 19*].

We found *H. danilewskii* of the normal type in a few small birds ; but *H. relicta* was not seen by us.

We wished to examine the blood of a large number of the civil population in order to estimate the percentage of them in which the parasites are to be detected ; but found such an investigation impracticable.

8. Methods for ascertaining Definitive Hosts.—The long researches of one of us in India, followed by those of Koch, Daniels, and the Italian investigators, have given us a very exact knowledge of the life-history of the Hæmamœbidæ in gnats, and have shown us how to detect them in the insects with ease and certainty. It has been noted that in inhospitable species of gnats the ingested parasites perish within the stomach cavity ; whereas in hospitable species the zygotes escape from that cavity and develop in the tissues, ultimately giving rise to blasts which are found in the juices and salivary glands of the insect. Hence to incriminate a given species of insect with regard to the human Hæmamœbidæ, it is necessary only to find the parasites within that species. In short, the method to be adopted is precisely that used in ascertaining alternative hosts for any other metoxenous parasite, such as Cestodes and Filaridæ, for instance. Care only is required to determine for certain that the parasites found in the gnats were derived from human beings, and not from other vertebrates. It is not necessary actually to infect human beings from infected gnats, because the general law that infection can be so produced has already been sufficiently established.

Hence a given species of insect is incriminated simply if we succeed in finding the parasites—

(1) in insects bred from the larva and fed on infected persons.

(2) in any insects fed on infected persons—provided that the zygotes found in the insects are of the proper stage of growth ; or

(3) in a large percentage of insects caught in a chamber where an infected person lives—provided that the circumstances exclude the possibility of the insects having become infected from other vertebrates.

9. Definitive Hosts in Freetown.—Our first object being the search for definitive hosts, we propose to record our operations in this connection in some detail.

For the reasons given in paragraph 22, suspicion was already strongly excited against all species of gnats of the genus *Anopheles* ; and, accordingly, we began by searching for these insects. Two species were found almost at once, under the following circumstances.

Dr. Berkeley, medical officer of the Lunatic Asylum at the eastern suburb of Kissy, informed us that there had been a small outbreak of fever among the permanent inmates of that institution. Three persons had been attacked; one had died, one had recovered, and one was convalescent. He was also good enough to bring us a number of gnats which he had caught within the institution. They proved to belong, almost all of them, to *Anopheles*; and were of two species, one a small species which we have named *A. funestus*, and the other a larger species called *A. costalis*, Loew. The former were by far the more numerous. [*Addendum I.*, *Plate V.*]

The insects brought by Dr. Berkeley were dissected and examined. In one a single characteristic zygote containing the melanin of *H. vivax* was found; and since the melanin of this species is typical of it, it was scarcely open to doubt that the zygote had been derived from that human parasite.

No further positive results, however, were obtained from this locality. No fresh cases of fever occurred, while we failed to find parasites in a large number of the *Anopheles* subsequently brought from the asylum. It was, however, interesting to observe these insects in the building. Large numbers of the small species were found gorged with blood and seated asleep in their characteristic attitude on the walls. They were most numerous on the ground floor and in the sleeping chamber of the inmates—who were not provided with mosquito nets. Few other gnats were present.

In the meantime our attention had been called to the fever so prevalent among the troops at Wilberforce. On searching the barracks and hospital we found a considerable number of the large variety of *Anopheles*, namely, *A. costalis*, already observed in small numbers at Kissy. As at the asylum, they were seen gorged and asleep in their characteristic attitude on the walls of the buildings, especially in dark corners, and were easily captured in test-tubes. Some were so excessively gorged that they had been evidently willing to fly only to the nearest part of the walls, just over the bed-heads of the soldiers on whom they had been feeding; and those which were at once examined were found to contain human blood—or at least blood microscopically indistinguishable from human blood.

The men—of whom about twenty slept without nets in each barrack—complained of being bitten by these insects at night, but added that they were occasionally annoyed also during the day, especially toward evening. It should be added that no other species of gnat were present within the barracks. During our stay in Freetown we must have caught or obtained at least two hundred gnats from these buildings, but only in a single case—a *Culex*—did the captures prove to be other than *A. costalis*. We noted also that there were absolutely no vertebrates other than human beings within or in proximity to the barracks. Cattle, dogs, cats, bats, monkeys were absent, while, owing to the buildings having been newly erected, there were not even sparrows, lizards, etc.

The *Anopheles* caught by us in test-tubes—almost all of them females which had evidently gorged themselves on the men during the preceding night—were reserved for microscopical examination. They were kept alive for 48 hours or more, until their meal of blood had been so far digested or voided as to permit an easy examination of the tissues of the stomach; and were then killed and dissected—the stomach, the thorax, and the salivary glands being especially

scrutinized. During our stay, altogether 109 fed female *Anopheles* caught within the Wilberforce Barracks were examined in this manner.

It has been stated [*paragraph 7*] that Hæmamœbidæ of all three human species had been found in about 25 per cent. of the soldiers at Wilberforce. It was therefore to be expected, if the *Anopheles* caught in the barracks were an hospitable species, that the parasites should be found also in a large percentage of these insects. And this proved to be the case. Out of the 109 insects examined the parasites were detected in 27.

The parasites were in all stages of growth; from the very youngest zygotes, evidently derived from the last meal made by the host; to the mature zygotes derived from previous meals, and the blasts lying in the salivary glands ready to produce fresh infections of human beings. We even judged it possible, in some of the dissections, to distinguish the different species of the parasites by their morphological characters [*paragraph 10*]. The following list, giving the serial number, and results of examinations of each insect in which the parasites were found (the negative insects being omitted) will be instructive :—

No. 4. Three nearly mature zygotes. Species unknown.
„ 8. One very small zygote, apparently *H. malariæ*, among stomach cells.
„ 12. Eight nearly mature zygotes. Species unknown.
„ 13. Six mature zygotes. Species unknown.
„ 14. Four very small zygotes, evidently of *H. vivax*.
„ 17. Several mature zygotes, one containing melanin, probably of *H. vivax*. Also empty capsules. Salivary glands loaded with blasts in cells and ducts.
„ 21. Eleven empty capsules (old) attached to stomach. One half-grown zygote. Salivary glands packed with blasts within capsule, cells and ducts.
„ 23. Eight very small zygotes of *H. malariæ*.
„ 29. Ten zygotes of *H. malariæ* of 3-4th day.
„ 31. Several large zygotes.
„ 32. Empty capsules. Blasts in salivary cells and duct.
„ 43. Three very young zygotes of *H. præcox*.
„ 56. Four young zygotes of *H. vivax*.
„ 57. Two young zygotes of *H. malariæ*.
„ 61. Several very young zygotes of *H. præcox*.
„ 62. One medium zygote of *H. vivax*.
„ 63. Several nearly mature zygotes, probably *H. malariæ*.
„ 65. Several smallish zygotes of *H. malariæ*, and one very young one of *H. vivax*.
„ 71. Several medium zygotes of *H. malariæ*.
„ 76. One empty capsule. A few blasts in one lobe of gland.
„ 79. One medium zygote with dark pigment.
„ 84. Three mature zygotes.
„ 89. One empty capsule. No blasts found (poor dissection).
„ 91. Six mature zygotes.

No. 92. Very numerous large zygotes, probably *H. malariæ*.

„ 99. Several empty capsules. Numerous blasts in blood, and a few in cells of salivary gland—none in ducts.

„ 108. One nearly mature zygote.

It is possible that the parasites may have been overlooked in some of the gnats examined. That a larger percentage of the gnats should not have been infected, and that more zygotes should not have been found in them, was to be expected from the old nature of most of the fever cases among the men [*paragraph 19*], and from the constant use of quinine by the medical officer.

The facts obtained, however, removed all possibility of doubt as to *A. costalis* being hospitable to the human parasites; and it was no longer necessary to undertake formal feeding experiments. Indeed the condition of things found in the Wilberforce Barracks amounted to an experiment of this kind being performed by nature herself under our eyes. This was the more fortunate as we experienced great difficulties in the way of actually feeding gnats on infected persons in a satisfactory manner. Nevertheless a few such experiments were performed.

On the 17th August, five small *Anopheles* (*A. funestus*) caught at Kissy, were fed on a case containing a few *H. malariæ*. The insects had been naturally fertilized and fed when caught, but had been kept for some days until they had digested their meal and had laid their eggs. They were then fed again *in test-tubes* on the case referred to, and were kept for another two days before being examined. Two yielded bad dissections, and one died before its meal of blood was digested. The fourth was negative. The fifth was dissected on the 19th, and was found to contain three very young zygotes with pigment identical with that of *H. malariæ*.

A few days later, one large *Anophele* (*A. costalis*), also caught by hand and previously naturally fertilized, fed, and kept in the same manner as the preceding, was re-fed on the same case. Two days afterwards several young zygotes containing the melanin of *H. malariæ* were found in it.

In both these successful experiments the insects concerned belonged to large batches of *Anopheles* procured from a building where there was no fever; and numbers of insects of both batches had been examined and found free from parasites. Judging from this fact, and from the *size of the zygotes found*, there could be no doubt that the latter had been derived from the case of *H. malariæ*. It should be carefully noted, however, that both these insects had evidently been fertilized before they had been caught, and had indeed each laid a batch of eggs before the experiments.

The military medical authorities now put a stop to our feeding any gnats, except those bred from the larva, on their cases. As it took us some weeks to obtain *Anopheles* in this manner, and as we could then only feed the insects in a very unnatural manner, that is, in tubes, our experiments in this line were practically arrested.

Altogether eighteen *Anopheles*, mostly of the large variety, all of them bred from the larva, and some of them bred from the eggs of *Anopheles* caught at Wilberforce, were applied in test-tubes to the skin of patients containing a few *H. præcox* (crescents) and *H. malariæ*, and *taking quinine*—no better cases being available. Being isolated in test-tubes when fed, none of these insects

were fertilized, while they could not be induced to feed copiously. All proved negative as regards the parasites. As, however, it was already certain that all the insects belonged to hospitable species, this result was interesting as tending to show that *cultivation experiments may fail if not performed under natural conditions.* The subject is discussed later [*paragraph 20*].

After our departure, Dr. Fielding-Ould examined numerous *Anopheles* caught at Wilberforce, and in the houses of natives suffering from fever, and found the parasites in 18 %. His observations on *Culex* were negative.

We made no observations on *Culex* or other insects.

We conclude that both *Anopheles costalis* and *Anopheles funestus* are hospitable to the human Hæmamœbidæ. We think that *A. costalis* is hospitable to all three of the human species; and *A. funestus* certainly to *H. malariæ*, and probably to *H. vivax*. But we made no observations regarding the connection of the latter with *H. præcox*.

10. The Hæmamœbidæ in the Definitive Hosts.—The stages of the human parasites in *Anopheles* are essentially the same as those of *H. relicta*, first described by one of us in a gnat of the *Culex pipiens* type—a fact already recorded by Koch and the Italian observers. In Plates I., II., and III. we give a series of photographs of all these Hæmamœbidæ in the gnat.

The following small differences between the human species and *H. relicta* were noted by us :—

(1.) The capsule of the zygotes of the human species is somewhat thinner; so that the youngest zygotes are more difficult to detect, and sometimes rupture under pressure of the cover-glass—a thing which rarely happens in *H. relicta*.

(2.) The mature zygotes seem to contain more blasts than do those of *H. relicta*.

(3.) The melanin appears to remain longer in the human species.

(4.) The blasts are shorter and thicker, and less bent and twisted, than those of *H. relicta*—but differences in the strengths of the salt solutions used may account for this.

(5.) In the human species the blasts tend to lie in the salivary cells in bundles, like bundles of faggots, and not irregularly like those of *H. relicta*—at least this was very noticeable in two of the gnats, numbers 17 and 99.

(6.) Owing to the dilatation of the proximal ends of the salivary ducts in *Anopheles*, the blasts can be more easily distinguished within these channels.

It is important to note that, though elements like those provisionally called "black spores" by one of us were frequently seen in the Freetown *Anopheles*, they were never detected within the capsule of the zygotes [*paragraph 24*].

The young zygotes of *H. vivax* were always immediately recognisable by the numerous minute granules of light brown melanin which they contained. The youngest zygotes were, in fact, almost identical in appearance with the mature gametocytes in the human blood. As the parasites advance in growth, the melanin seems to decrease in quantity and become slightly paler; but it was sometimes still to be found even in the most mature zygotes—which, moreover, were of very large size, and contained very numerous blasts.

The zygotes of the other two species contained fewer and much darker granules. In the youngest ones we considered we could distinguish between the melanin of *H. malariæ* and that of *H. præcox ;* the former possessing large and small granules of a dark brown colour, mixed together ; the latter possessing only large black granules.

Our studies were not sufficiently exhaustive to enable us to speak regarding other differences noted by the Italian observers. Nor had we time to examine cytological details—a thing to be regretted, because the Romanowsky method does not seem to have yet been correctly applied to the study of the nuclear changes in the zygotes. We easily noted, however, the structures first described in *H. relicta ;* namely, the alveolar, vacuolated appearance of the zygotes; the denser chromatoid granules ; the division of the parasites into 8—12 meres, giving rise to spherical blastophores carrying blasts in a manner recalling spermatogenesis ; and the minute spherical enlargements often attached to the immature blasts. We observed also that in the human species, as (to a less degree) in *H. relicta*, zygotes which are little more than half grown, often contain apparently mature blasts.

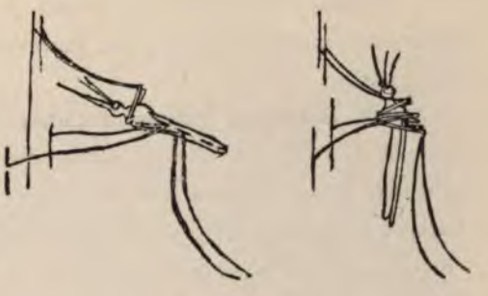
In several insects many full-sized zygotes appeared not to have ripened—that is, did not contain blasts ; although others containing blasts were found associated with them.

IV. BIONOMICS OF THE CULICIDÆ IN FREETOWN.

11. Differences between *Culex* and *Anopheles*.—Our next duty was to study the habits of the inculpated *Anopheles* with reference to the prevention of gnat-fever. We propose to give our bionomical observations here,* and to reserve a description of the insects themselves for the Addenda.

In view of the particular connection which seems to exist between *Anopheles* and gnat-fever, it is important to possess information sufficiently simple to enable anyone to distinguish between them and the common gnats of genus *Culex*. Such points of difference have already been recorded by one of us as the result of his observations in India [14] ; and we were able to assure ourselves that the same differences hold also in Freetown.

(1). *Adults*.—The principal zoological distinction between *Anopheles* and *Culex* is that the *palpi* of the female are long in the former and short in the latter. But this is of little assistance to those who are not entomologists, and who may not know what the palpi are. Fortunately there is a more striking difference—one which can be perceived by anyone at a glance—in the *attitude* adopted by the respective insects when seated on a wall. In *Anopheles* the axis of the body is almost vertical to the wall ; in *Culex* it is parallel to the wall. Popularly put, the *Anopheles* may be said to stand on its head, with the point of its tail projecting outward ; while the tail of *Culex* points downward, or even a little toward the wall.



3. *Anopheles* and *Culex*.

Another noteworthy point of difference is the general *shape* of the body. In *Anopheles* the head and thorax are comparatively small, while the long palpi are held in contact with the proboscis. This gives the insect a peculiarly elegant fusiform shape—like that of a humming-bird moth ; while the proboscis looks like a long thick beak. On the other hand, *Culex* has a thick ungainly thorax, and a thin bare proboscis. In short, the appearance of *Anopheles* suggests that it can fly further and more rapidly than *Culex*.

Thirdly, the wings of *Anopheles* are generally spotted along the anterior edge (to use popular

* It should be clearly understood that our remarks apply only to our own observations. We cannot answer for facts in other localities.

language), while those of *Culex* are generally plain. There are exceptions to both these statements; but the rule holds good in Freetown, and also, according to the observations of one of us, in India.

The eggs of *Anopheles* have a peculiar boat-like shape, while those of *Culex* are oval or lanceolar.

(2.) *Larvæ.*—Here too, very fortunately, there is a marked difference. The *tracheæ* of *Culex* terminate in a long buoy-like breathing tube which projects, sometimes to a great length, above the tail fins. On the other hand, the tracheæ of *Anopheles* merely terminate just above the tail fins, in two apertures which are nearly flush with the back.

Apparently owing to these facts, the *attitude* also of the larvæ of the two genera is different: those of *Culex*, when at rest, hang suspended head downwards by the attachment of their breathing tube to the surface of the water; those of *Anopheles* float flat on the surface like sticks.

When disturbed, *Culex* larvæ immediately leave the surface and wriggle to the bottom of the water, floating up again a few moments afterwards by the buoyancy of their air-tube. The movements of *Anopheles* larvæ are quite different. They move on the surface of the water with a few peculiar backward jerks, followed by a pause; and sink to the bottom only when much molested.

Lastly, *Culex* larvæ live mostly in *artificial* collections of water, while *Anopheles* larvæ prefer *natural* collections—a point, however, which requires special consideration.

12. Breeding Places of *Culex*.—In Freetown, as in all collections of houses, there are to be found in the vicinity of human dwellings innumerable tubs, pots, shards, etc., in which a little rain water or household slop water collects. In almost all such in Freetown—tubs, pots, buckets, cisterns, empty oil-tins, biscuit-tins, sardine-tins, gourds, flower-pots, broken pottery, crockery, and bottles, even in unbroken bottles thrown uncorked on heaps of refuse—we found *Culex* larvæ. The only condition necessary to their existence appeared to be an ounce or more of water preserved from desiccation or leakage. Occasionally, also, the larvæ were found in hollows in rocks, pools by the side of roads, and even in small runnels of water. Very numerous larvæ of a gnat of the *C. tæniatus* type were observed in hollows in rocks on the top of Signal Hill (400 feet), near the Signal Station (occupied); also some of the same larvæ, in a hole in rock hundreds of yards from human habitations.

Similar facts have been always observed by one of us in India. Drains, ditches, and small ponds must be added to the haunts of *Culex* larvæ; but, as a rule, vessels of water near houses appear to be their favourite habitations.

13. Breeding Places of *Anopheles*.—We made a very elaborate search in Freetown for *Anopheles* larvæ. Every part of the town shown in a map which was given to us was carefully examined; and wherever a breeding place of *Anopheles* was observed it was entered on the map [*Map II.*].

We recorded the following observations :—

(1.) *Anopheles* larvæ were never found in vessels of water, except in a single instance—a tub.

(2.) A mangrove swamp fringing a part of the coast (Aberdeen Creek), far from human habitations, contained no larvæ, although there were numerous fresh-water pools on the edge of the dry land. Small fish abounded in the swamp.

(3.) Part of a large swamp below Wilberforce Hill, and also one at Kissy, was examined by ourselves, assisted by several natives. Water lay to the depth of an inch or two between clumps of grass, on a basis of flat rocks. Habitations were distant. No larvæ found.

(4.) Owing to the hilly nature of Freetown there are not many ponds or large pools. Such as there are contained no larvæ—although in several of them there were no fish.

(5.) Rapid streams contained no larvæ.

(6.) Pools left drying after rain in watercourses, or other positions liable to scouring by heavy rain, seldom contained larvæ—at least at the season when we were at Freetown.

(7.) Shallow puddles of rain-water, apt to dry up between the showers, seldom contained larvæ.

(8.) Puddles which lasted from shower to shower, but which were not liable to be scoured out by heavy rain, generally contained them.

(9.) Pools on soil or rock fed by water oozing from the ground, and small slow runnels of water amongst herbage, usually contained large numbers of larvæ—unless liable to scouring.

Altogether we counted within the area of the map about one hundred principal pools containing *Anopheles* larvæ.

Generally speaking, we found the larvæ in *small permanent pools not liable to scouring*. We did not find them in *vessels, evanescent pools, pools liable to scouring, large collections of water, and running streams.*

It would appear, then, that the larvæ require certain conditions, namely, security from (1) desiccation, (2) scouring. To these should perhaps be added (3) security from small fish.

4. A large *Anopheles* pool in roadside ditch.

As may be surmised, then, *Anopheles* pools were not found on *sloping ground*—where puddles either dry up very quickly or are apt to be scoured out by heavy showers. On the other hand they were frequently found on *level ground*. We did not find them on the hills surrounding Freetown (though they were probably present in a few places) ; we did find them frequently in the flattest and lowest parts of the town.

The majority of the *Anopheles* pools were found in the ditches by the side of roads and in certain localities where many small streamlets ooze from the bases of the hills after rain. Others existed in the course of the newly-made railway. Few were observed in the yards and gardens of houses.

5. Large *Anopheles* pool produced by railway embankment.

Most of them contained green water-weeds, and many tadpoles and frogs; but none contained fish.

In size they varied from a few square inches in area to several square yards.

It is important to note that in no case did we find *Anopheles* larvæ in pools which, though otherwise suitable for them, were distant from human habitations. We must add, however, that we did not examine many such, except in the swamps referred to above.

In considering these facts it is necessary to remember two things. *First*, Freetown is a hilly place with a low subsoil water—circumstances which limit the number of surface puddles of all kinds, and especially of permanent stagnant puddles. *Secondly*, when we carried on these investigations it was the height of the rains; that is, heavy showers usually fell several times a day. This, in a hilly country, must act in opposite ways as regards surface pools. It constantly replenishes all these pools, but at the same time it constantly scours out those which exist in watercourses and on sloped surfaces. A different state of things may prevail as the showers become less frequent toward the end of the rains; the smaller puddles will then tend to dry up between the showers, while those formerly subject to scouring may now cease to be so, and may become habitable for *Anopheles* larvæ. Thus we thought it likely that after the rains they may be found in the numerous pools which will then be left in the beds of the drying-up watercourses—where, during the rains, they would be swept away.

Hence it is not advisable to generalize too much from these observations, regarding either the frequency of *Anopheles* puddles in other places, or even in Freetown at other seasons. In some places the puddles must be more numerous; in others less numerous; while in Freetown itself their number and position probably change from season to season.

6. Collection of small *Anopheles* pools.

14. Distribution of *Anopheles* in Freetown.—Owing to difficulties in the way of catching gnats in private houses, we could not make exhaustive studies of the distribution of the adults in various parts of the town. The following facts, however, were observed:—

(1.) *A. costalis* was found all over the low-lying areas examined by us. That is, the larvæ which yielded this species were found in all these areas; though it was much more difficult to obtain the adults. The adults were most numerous in the Wilberforce Barracks (600 feet); but were also obtained in other parts of the town, as far east as the suburb of Kissy. No adults were ever seen in the barracks and officers' quarters on Tower Hill (400 feet); although there were numerous breeding-pools only 500 yards to windward. None were found in the barracks on Mount Aureole (900 feet); although pools lay all round the base of the hill.

(2.) *A. funestus* was restricted entirely to the eastern part of the town; we never found a larva nor an adult of this species west of Government House. Not one was seen in Wilberforce Barracks, where *A. costalis* was so common. Individuals were caught in a house in the town where some cases of fever had occurred; at Leicester, a village high among the hills; and at some other points. The larvæ were common in pools in the eastern quarters, at Kissy, and other spots.

It is curious that while only the large species occurred at Wilberforce, it was *almost* entirely the small species which was found at Kissy.

15. Bionomics of *Anopheles* Larvæ. We made the following observations:

(1.) *Eggs.* These are *boat-shaped*, like those of *Anopheles* observed in India. They appear to be laid singly on water; but cohere by their ends, forming typical triangular patterns; and also adhere to floating objects, the sides of the vessel, etc. We observed no facts indicating that they are ever laid on solid surfaces. *In vitro* they take about 24 hours to hatch; but the period is probably much shorter in puddles.

(2.) *Duration of larval stage.* This depends on temperature and amount of food. Under natural conditions it may probably be only three or four days; but under unfavourable conditions (cold, overcrowding, absence of food) it may certainly extend to weeks.* There are reasons for thinking that development is much hastened by bright weather, in order to enable the imago to hatch out before desiccation of the containing puddle.

(3.) *Food.*—The larvæ were frequently watched floating on the surface and feeding on filaments of water-weed—amongst which they often entangle themselves. On dissection, the intestine was found crammed with these filaments. It was observed that *in vitro* the larvæ scarcely grow in size unless they are given large quantities of water-weed—which they dispose of very rapidly. On the other hand, larvæ were often caught in puddles in which no green vegetation could be seen. They may eat other food, but it would seem as if water-weeds constitute their favourite diet. It was also noted that they obtain shelter among these weeds from the current running through the pools during or after rain.

(4.) *Enemies.*—No observations could be made under this head; but we often found many frogs and tadpoles in the breeding-pools, apparently living at peace with the larvæ.

(5.) *Effects of desiccation.*—During most of our stay in Freetown heavy showers fell several

* One of us kept *Culex* larvæ alive for two months in a bottle in the cold weather in India.

times a day, so that the larvæ could live secure from desiccation in all but the most evanescent puddles. In September, however, there was a complete break in the rains, lasting three days. A large number of the pools, even many of those containing water-weed, and those fed by springs during rain, dried up completely. The question whether the larvæ had the power of living in the mud at the bottom of the pools could now be tested by direct observation. The break in the rains was followed by heavy showers, which immediately refilled all the puddles. Had the larvæ continued to exist in the mud, they would now have emerged again. As regards the puddles in which the mud had completely dried, this was not the case. No larvæ at all were found in them for at least two days after the rain had refilled them. After that interval larvæ again appeared; but they were very small ones, evidently just hatched from the egg. On the other hand, it was frequently observed that if the mud did not become completely dry, the larvæ would emerge into active existence after another shower. These observations were supported by some experiments *in vitro*; and we therefore conclude that the larvæ can withstand partial, though not complete, desiccation.*

(6.) *The same puddles constantly occupied.*—We have suggested [*paragraph 13*] that the position of the breeding pools may change according to the seasons; but while we were in Freetown there was no change of season, and we generally found *Anopheles* larvæ in the same puddles—namely, in those which were suitable for them. Thus, of two puddles lying close together, one would never contain larvæ, and the other would always contain them. The explanation of this probably is that the larvæ perish in the unsuitable pools, or that the adults generally return to the same pools in order to lay their eggs. It seems likely that the adults generally lay their eggs in the pools in which they themselves were bred; and that the insects thus learn by experience the places most suitable for them.

(7.) *Detection.*—It is easy to overlook *Anopheles* larvæ unless they are searched for in a bright light.

(8.) *Pupæ.* The pupæ of *Anopheles* seem to be smaller than those of the commoner species of *Culex*. They require about 48 hours to reach maturity *in vitro*; perhaps less in natural conditions.

16. Bionomics of Adult *Anopheles*. (1.) *Hatching.*—The adults generally hatch out in the evening; but their exit seems often to depend on the meteorological conditions of the moment, and appears to be delayed by rainy and windy weather.

(2.) *Food.* They can easily be kept alive in glass cages, test-tubes, bottles, etc. We kept some in this manner for a fortnight, and could doubtless have kept them longer if we had wished to do so. We were able to confirm Bancroft's statement [18] that gnats feed on bananas; but they seem to prefer the fresh fruit. During the day the insects remained at rest on the walls of the cage; but in the evening began to fly about and to walk over the fruit, plunging their proboscis into it in many places, so that the banana was sometimes covered with the gnats, both

* One of us reared adults from full-grown larvæ kept on damp blotting-paper (in India); but found that the young larvæ died when kept under these conditions.

male and female. They also drink water frequently, and can often be seen to be distended with the fluid. Raw meat was offered to them, but they could not be observed to touch it. Earth placed at the bottom of the cage seems to be suitable for them.

According to the accounts of the soldiers at Wilberforce, they bite almost entirely in the evening and night, but have been known to feed on men during the day. They can certainly be fed on men artificially during the daytime, simply by placing them in test-tubes and then applying the mouth of the tube to the skin. The stomach can be observed to become distended in from one to two minutes or more; after which the insect continues to suck, but commences to evacuate by the anus serum containing a small percentage of red corpuscles. *Culex* voids only a clear fluid under the same circumstances. The insects sometimes continue sucking like leeches for five or ten minutes, voiding blood all the while; but at other times soon withdraw the proboscis and then try another spot. It was noted, however, that *Anopheles* fed in this manner, even after they had remained sucking for five or ten minutes, never showed any great distension of the abdomen; while the contents of the stomach still remained for some time transparent and red as seen through the scales of the living insect. Moreover, in these cases the meal was generally digested or voided within about 24 hours.

On the other hand, *Anopheles* which had fed themselves under natural conditions generally presented a very different appearance. They were enormously distended; while the contents of the stomach were thick, opaque, and black, and sometimes did not disappear for three days. The only inference is that, under natural conditions, the insects which can manage to do so gorge themselves over and over again during the night—probably from the same subject.

(3.) *Propagation.*—We also observed that while naturally-fed gnats *invariably* laid eggs after two or three days, those which had been bred from the larvæ in captivity, and had then been isolated and fed in test-tubes, *never* did so, although before being isolated they had long been in company with males. The inference is that fertilization takes place only after the female has been fed.

We noted also that, in a cage where many male and female gnats, both *Culex* and *Anopheles*, were kept together for weeks, eggs were never laid—although the insects were fed as described on bananas, and the cage contained water for them to lay their eggs in. It seems, then, that a meal of blood is necessary before fertilization.

Lastly, we observed that previously fed and fertilized insects would lay a second batch of eggs after a second meal of blood, without a second fertilization; but never laid a second batch of eggs without a second meal of blood. That is, one fertilization suffices for several batches of eggs, but one meal of blood for only one batch of eggs.

These observations are wholly in accord with the results of the prolonged study of many kinds of gnats made in India by one of us; and it therefore seems likely that the following law is likely to hold good for the *Culicidæ* which feed on men—at least for the commoner species:—

Although these gnats can live indefinitely on fruit and perhaps juices of plants, the female requires a meal of blood, both for fertilization and for the development of her ova. In other words, *the insects need blood for the propagation of their species.*

Blood was never found in male *Culicidæ* in Freetown—according with the general law.

(4.) *Haunts.* The large majority of *Anopheles* caught by us in dwellings were females which were generally much gorged, and, if fed at all, were invariably fertilized; in other words, the males and unfed, or only slightly fed, females did not generally remain in the houses during the daytime, or if they did remain, kept in the roofs or other dark places where they were little observed. On the whole, we think that only those females which are so gorged that they cannot fly far remain in the houses during the day. We observed that if a cage full of *Anopheles* was disturbed in the daytime, the insects always struggled toward the light as if to fly out from the windows; and several which escaped from the cages actually did so. On one occasion a large number escaped from their cage during the night in the rooms occupied by one of us—none of them could be seen next morning.

Yet we may be quite sure that both the males and the unfed females haunt the houses during the night. The invariably fertilized condition of the gorged females caught in the houses shows that the males must be present in the houses when the females feed—since the latter are often so much distended after feeding that they are obviously unwilling to fly even a few feet from the bed of their victim; in other words, fertilization must take place within the houses. The unfed females must, of course, resort to human habitations during the night in order to obtain their food at all.

These facts would seem to indicate that in Freetown, in the rainy season, the *Anopheles* resort to the houses during the night, but that all except the gorged females live elsewhere during the daytime—possibly sleep in the trees and shrubs. The point is of interest as tending to show that large numbers of *Anopheles* may be present in a dwelling during the night, without it being easy to find them during the day.

It should, however, be added, that in India males and unfed females were often found in the houses in large numbers by one of us. Possibly different species have different habits in this respect.

Several old residents of the country informed us that gnats are usually very prevalent in the presence of much vegetation—especially long grass and undergrowth. Though it is difficult to see how such can favour the larvæ, we can understand that much vegetation can shelter the adults of certain species, which may even feed on particular kinds of plants when they cannot obtain blood, and may consequently find it easier to live where these plants afford them both food and shelter than elsewhere. It must also be remembered that gnats can certainly bite birds and other mammalia besides man; and that such are apt to congregate where there is much vegetation. On the whole, then, there is nothing improbable in the idea that the Freetown *Anopheles* should live outside the houses in the daytime.

(5.) *Proximity of breeding-pools to houses.* The gorged females are, as we observed, always fertilized, and must therefore probably lay their eggs a few days later or perish. We often noticed that gravid females kept in dry test-tubes made no attempt to lay eggs unless a drop of water was put in—in which case they often deposited their eggs almost at once in the fluid. These observations suggest the conclusion that the females must return to water a few days after each

meal of blood. As *Anopheles* larvæ were not found in vessels of water close to houses, the only inference which remains seems to be that the pregnant females must return, not to *any* water, but to some pool suitable for her offspring. If such a pool exists close to the house where she obtained her meal of blood, her journey need only be a short one; but if it be at a distance, she must fly correspondingly far—and may often be obliged to do so against the wind, or through heavy rain, or between trees.

It is therefore highly probable that, unless they can find animals and birds to feed on, *Anopheles* must seek their food in houses which are as near as possible to their breeding-pools, and also, *vice versâ*, select pools for their eggs which are as near as possible to the places where they obtain their food. In other words, they are not likely to neglect adjacent houses and pools in order to visit more distant ones. Of course many individuals must go astray—must be carried away by winds and so on; but it seems reasonable to expect that, as a rule, the breeding-pools and feeding-grounds will be as close together as possible.

Where necessity compels, however, it is probable that *Anopheles* do travel considerable distances to and fro. This must especially be the case during the dry season, when the pools must be much more rare—unless we can show that at that time the surviving adults cease to feed at all and "hibernate."

It is noteworthy that in the Tower Hill barracks we never found *Anopheles*, although there were numerous breeding-pools at a distance of only 500 yards, both to leeward and windward. The barracks are situate on a hill only 400 feet above the sea, and are surrounded by open grass, over which the insects could fly or be carried by the wind; though it should be added that occupied houses intervene between the pools and the barracks. Perhaps the insects are all attracted by these houses. The same thing was observed regarding the barracks on Mount Aureole.

The question as to the maximum distance possible between a house and a breeding-pool is an important one, but difficult to reply to. The distance will probably depend largely on local conditions, prevalent winds, screens of trees, presence of other animals, etc. We must remember that while wind may assist the insects going one way, it may equally oppose their return. On the whole it appears difficult to imagine that this maximum distance can be very great—more than half a mile, for instance; and certainly we never found *Anopheles* larvæ except in much closer proximity to houses.

Certainly gnats can visit ships lying fairly close to the shore. Dr. Wigglesworth was kind enough to give us some gnats which he had caught on board the S.S. *Fantee*, on the West Coast of Africa; and one of these was an *Anopheles*. But it must be remembered that insects blown across water are likely to travel much further than on shore—where hills, trees, and houses intervene to afford them rest and shelter.

If large numbers of *Anopheles* are found really far from human habitations, it will be not unreasonable to suppose that they can procure other besides human food. *Anopheles* are known to feed on horses and cattle, and may possibly bite other mammals, birds, and even reptiles. But it must be remembered that, in towns and villages at least, men are generally the commonest vertebrates present.

Owing to their habit of breeding in the vessels of water which generally abound near houses, the pregnant females of *Culex* need not fly so far to find water suitable for their eggs. It is possibly owing to this fact that, to judge by their more clumsy shape, they are less adapted for prolonged flight than *Anopheles*.

(7.) *Not attracted by lamps.* It is often thought that gnats are attracted by lamps and candles, and perish in them. They may be attracted to houses by lights burning in the windows; but we have never seen them hovering round the flames as moths and other insects do. They seem to bite less readily when there is a light in the room.

(8.) *Act of puncture.*—On applying *Anopheles* in test-tubes to the skin we observed that the insects would sometimes make puncture after puncture without drawing any blood. In such cases however, each puncture was invariably followed by the usual weal—showing that the insect always commences work by injecting some of its saliva. It therefore follows that an *Anopheles* may inject some of its saliva (possibly containing the blasts of the Hæmamœbidæ) almost instantaneously that is, before the victim feels the irritation and drives the insect away.

It is certain that one can be bitten without knowing it—especially during sleep. Thus one often finds gorged gnats within the mosquito netting in the morning—without having been at all conscious of their bites during sleep; and it is therefore probable that those who sleep without nets may be much bitten without appreciating the fact. Even the weals often disappear in an hour or two; so that not even the marks of the punctures need necessarily remain until next day.

One of us thinks that much less irritation is caused if the insect is allowed to suck its fill, and has frequently observed the fact on his own person. This may be due to much of the irritating saliva having been sucked back during haustellation.

From several accounts it would appear that *Anopheles* hover over their victim less than *Culex* do, and consequently do not attract his attention so much by humming.

People often imagine that gnats will not bite them; but it seems to us impossible to accept such statements without sufficient experimental proof. What is more likely to be the case is that some persons do not much feel the punctures. The skin of some persons is not very sensitive; and it is moreover possible that immunity may be acquired against the irritation of the injected saliva. Thus persons who have recently arrived in the tropics are well known to suffer more than old residents. This does not necessarily mean that the latter are less frequently bitten, but, possibly, only that they have become comparatively inured to the irritation.*

17. Bionomical Questions still requiring Investigation.—The facts about gnats hitherto collected by no means exhaust the subject. We will indicate some of the more important questions which we found it impossible to deal with.

(1.) *Life in the dry season.* Between the rainy seasons many places in the tropics are extremely arid and free from pools suitable for *Anopheles* larvæ. How then do the insects manage to continue the species from one rainy season to the other? We may assume (1) that the larvæ

* It is however possible that the body may in the course of time produce some substance offensive to the insects, just as it may produce substances offensive to micro-organisms.

may continue to exist in such pools as do remain, the insects multiplying enormously after the first showers; or (2) that the eggs may lie dormant in mud; or (3) that the adults may "hibernate," or at least live on without feeding on blood at all, and consequently laying no eggs. We cannot pretend to give an answer to this question. In Italy it appears that the adults hibernate through the winter. One of us made an observation tending to show that the eggs can withstand desiccation for several months; but this requires confirmation. The subject appears to be of great importance in connection with the prevention of gnat-fever [*paragraph 28*].

(2.) *Other subjects.*—It is necessary also to examine how far *Anopheles* do or do not exist apart from human dwellings; whether they can breed in other collections of water than those mentioned above, as, for instance, in rain-water collected in leaves; whether they really have any connection with rank vegetation; how far they can spread from their breeding pools, how far they feed on animals, and so on.

We must again emphasize the fact that our remarks as to the bionomics of these insects are drawn only from our experiences within a limited area of Freetown [*Map II.*] in the months of August and September. We can make no statements regarding their habits, or the habits of other species of *Anopheles*, outside this limited area, or at other seasons.

18. **Source of the Wilberforce *Anopheles*.**—As an instance of the difficulties which may be met with, we may mention that, in spite of repeated efforts, we never succeeded in finding the source of the *A. costalis* which were so prevalent in the barracks at Wilberforce. These barracks are situated on a ridge 600 feet above sea level, and are surrounded by native houses and gardens, and by much rank vegetation. The ground slopes on either side down to the plains. Far below to the west (that is, to windward during the rains) there are a marsh and a mangrove swamp.

We searched in vain every spot likely to harbour *Anopheles* larvæ; and even examined the distant marsh and mangrove swamp without positive results. After our departure Dr. Fielding-Ould and Captain Smith, R.A.M.C., continued the search—equally in vain, except that they found larvæ in pools 1,500 yards away [*Addendum II.*].

It was often observed, however, that the *Anopheles* frequented two or three of the barracks in preference to the other buildings, and that these barracks were at the end of the block of houses. At the same time, the insects were common in the houses of the officers, who lived at that time in the village. These facts suggest that their source existed near these buildings, and not at any great distance, and that it was probably overlooked owing to the thick vegetation present. It seems very unlikely that such numbers of the insects could come from breeding pools in the marshes which lie thousands of yards away in the plain below.

The fact that so many of them were caught for examination seemed materially to affect their numbers; but we noted that newly-arrived insects invaded the barracks on one, if not more, occasions.

V. DISCUSSIONS.

19. Questions regarding the Parasites.—Having recorded our observations in Freetown, we propose next to consider some questions of importance in connection with our subject.

The life-history of the Hæmamœbidæ as now known, and as given in the terminology previously adopted by one of us in consultation with Professor Herdman [15], is as follows [*Plates I., II., III., IV.*] :—

The youngest parasites exist as *amœbulæ* or *myxopods* within the red blood-corpuscles of the vertebrate hosts. On reaching maturity they become either *sporocytes*, or *gametocytes*.

The sporocytes are destined for the asexual propagation of the organisms within the vertebrate hosts. They give rise by division to spores; which escape into the plasma, enter fresh corpuscles, and in their turn become amœbulæ—thus continuing the species indefinitely in the blood.

The gametocytes, however, are sexual forms, male and female—the male containing a number of *microgametes*; the female, one *macrogamete*. They remain unchanged within the vertebrate hosts; but on being drawn into the stomach of a suctorial insect, perform their sexual function. The male gametocyte emits its microgametes; one of which enters a macrogamete and fertilizes it. The fertilized macrogamete is called a *zygote*.

If the suctorial insect be a species hospitable to the parasite, the zygote next escapes from the contents of the stomach, pierces the wall of the organ, and affixes itself in or on the outer coat. Here it grows largely in bulk. Its substance divides into from about eight to twelve *meres*. Each mere becomes a *blastophore* bearing a large number of filamentous *blasts* affixed to its surface by one extremity. As growth advances the blastophores disappear, leaving the capsule of the zygote packed with the blasts. The capsule now ruptures, allowing the blasts to escape into the body-cavity of the insect. From this they work their way into the insect's salivary gland; and finally pass from this gland into the blood of a new vertebrate host, in which they eventually become the amœbulæ with which the cycle commenced.

As to terminology—it will be perceived that we use the word "spore" for the asexually-produced progeny of the parasites, and the word "blast" for their sexually-produced progeny. Originally, however, neither word possesses such restricted meanings: a "spore" is a single cell capable of separating from the parent and of starting by itself a new bion or individual; while "blast" simply means a bud. Considering, however, that the recent demonstration of typical sexual functions among the Coccidiidæ and Hæmamœbidæ originates the new case of such functions (apart from simple conjugation) among unicellular animals, it may be now justifiable

to employ these terms as indicated. We may admit that, strictly speaking, the blasts are also spores, while the spores are, in a sense, also blasts; but it will certainly be convenient in the present case to *apply* the word spore only to the asexually-produced progeny, and the word blast only to the sexually-produced progeny.

Obviously the zygote is the homologue of a fertilized ovum; and the blasts are homologues of the cells which spring from the fertilized ovum, and which construct the tissues of multicellular organisms. Only, in this case the blasts give rise, not to a cluster of cells, but to a cluster of distinct unicellular animals—by a kind of polyembryony in fact. Hence the use of the word "blast" for the progeny of the zygote appears to derive sanction from the homologous use of the same word in embryology. The word "zygote" seems to be acquiring the restricted meaning of the result of union of two similar gametes; we use it as often applied, namely, to express the result of union of any two gametes.

Of course other terminologies may be preferred by some. Professor Ray Lankester has recently been kind enough to suggest terms, which bear the weight of his authority and may be liked by many. He suggests "androspore" and "gynospore" for microgamete and macrogamete respectively—terms certainly much more definite; "gametospore" for zygote; "nomospore" for the asexually-produced spore; "oudeterospore" for any spore which is not differentiated so as to be male or female; and "gametoklasts" or "gametoblasts," or even simply "filiform young," for the blasts.

Manson was the first to interpret rightly the ascertained facts about the sexual propagation of the parasites, and his views have been adopted by other writers; but he has attempted no terminology. Grassi and Dionisi use "gamete," "microgametogene" (for microgametocyte), and zygote [19]. The term gamete had been previously applied by Schaudinn and Siedlecki to the case of some of the Coccidiidæ. The Italian observers also use the word "sporozooids" for the blasts.

Koch employs simply "spermatozoon" for microgamete [11]. The zygotes he calls "spherical bodies (Kugelförmige Gebilde)"; the meres, "secondary spheres"; and the blasts, "sickle-shaped bodies (Sickelkeime)."

The terms "sporozooid" and "sickle-shaped body" suggest somewhat doubtful analogies with the Coccidiidæ. The former term, moreover, is not very intelligible—sporozooids being, in fact, spores; while the so-called spores of the sporozoa are really often sporocysts. Compare also Mesnil [20].

The simple expressions ovum and sperm may be preferred by many to macrogamete and microgamete. Professor Harvey Gibson suggests "oosperm"—that is, the combined ovum and sperm—for zygote. The word "zooid" has been suggested in the place of blast; but zooid has the acquired meaning of a motile cell, and we do not yet know that the blasts are motile.

A biological point of some interest requires reference. Several writers seem to assume that the zygote in the gnat is the same individual organism as the female gametocyte in the vertebrate host—that it is, in short, the fertilized female parasite. This idea receives some support from the fact that the zygote contains the melanin of the gametocyte. On consideration, however, it

seems much more likely that the life of the individual gametocytes, both male and female, ceases with the sexual function in the cavity of the insect's stomach, and that at that moment the life of a new bion—the zygote—begins. The gametocyte and the macrogamete are not identical. The former contains the latter. Perhaps this fact is best indicated by the presence of those minute spherical appendages, which have long been observed in connection with the parasites after they escape from the enclosing corpuscle, and which Professor Herdman suggests may be the homologues of *polar bodies*. Thus the female gametocyte really contains several cells, only one of which, however, reaches full development as a macrogamete. Similarly the male gametocyte also contains several cells, only one of which becomes mature and gives rise to the microgametes. Hence it is not the female gametocyte, but the progeny of the female gametocyte, which is fertilized, and this fertilized progeny constitutes a new individual, which passes its existence in the tissues of the gnat. That the macrogamete retains a portion of the tissues (containing the melanin) of its parent gametocyte is not inconsistent with biological laws; but it should be noted that, according to Mac Callum, the zygote of one avian species of the Hæmamœbidæ actually does discard the melanin of the parent gametocyte.

Hence it would seem that the Hæmamœbidæ are organisms which exhibit the phenomenon of "alternation of generations." Their full life-cycle consists of two stages—one passed principally in the vertebrate host; the other entirely in the insect host.

Mannaberg's idea that two or more amœbulæ in one corpuscle conjugate, has found support from the observations of one of us in connection with *H. relicta*. It seems possible that the gametocytes are produced by such conjugation. At any rate, it is difficult to understand what else but conjugation can happen to two or more amœbulæ in this position.

It is very noteworthy that, while Europeans who come from the West Coast of Africa suffering from gnat-fever often contain large numbers of crescents, these bodies could scarcely be found at all in Freetown. This experience tallies with the observation of one of us in very malarious districts in India [21]. It would seem that gametocytes cease to be produced, at least in large numbers, when an infection has lasted a long time—as for instance more than six months or a year. This may be explained on the supposition that the number of parasites tends to diminish as a case advances, owing to gradual production of partial individual immunity, and that it is therefore rare to find two amœbulæ in one corpuscle in old infections. Of course, in very malarious localities the majority of cases are old infections; whereas patients who resort to the Tropical Ward in Liverpool are generally comparatively recent cases [22].

We have used the taxonomical nomenclature previously suggested by one of us [15]. It is as follows (inclusive only of the parasites of men and birds):—

Family: HÆMAMŒBIDÆ, Wasielewski.

Genus I. *Hæmamœba*, Grassi and Feletti. The mature gametocytes are similar in form to the mature sporocytes before the spores have been differentiated.

Species 1: *Hæmamœba danilewskii*, Grassi and Feletti. Syn.: *Laverania danilewskii*, Grassi and Feletti, in part; *Halteridium danilewskii*, Labbé; etc. Several varieties possibly distinct species. Parasite of pigeons, jays, crows, etc.

Species 2 : *Hæmamœba relicta*, Grassi and Feletti. Syn. : *Hæmamœba relicta* + *H. subpræcox* + *H. subimmaculata*, Grassi and Feletti ; *Proteosoma grassii*, Labbé ; etc. Parasite of sparrows, larks, etc.

Species 3 : *Hæmamœba malariæ*, Grassi and Feletti. Syn. : *Hæmamœba laverani*, Labbé, in part. Parasite of quartan fever of man.

Species 4 : *Hæmamœba vivax*, Grassi and Feletti. Syn. : *Hæmamœba laverani*, Labbé, in part. Parasite of tertian fever of man.

Genus II. *Hæmomenas*, gen. nov. Syn. : *Laverania*, in part + *Hæmamœba*, in part, Grassi and Feletti. The gametocytes have a special form (crescentic).

Species : *Hæmomenas præcox*, Grassi and Feletti. Syn. : *Hæmamœba præcox* + *H. immaculata* + *Laverania malariæ*, Grassi and Feletti ; *Hæmamœba laverani*, Labbé, in part ; etc. Several varieties—possibly distinct species. Parasite of the irregular, remittent, pernicious or æstivo-autumnal fever of man.

The gametocytes of *H. danilewskii* are crescentic, but they are still shaped like the sporocytes (which are also crescentic)—the shape of both being simply determined by the exigencies of space within the corpuscle. There appears, then, to be no sufficient reason for separating this species from the others of genus *Hæmamœba*, in which the gametocytes and sporocytes have the same shape. On the other hand, *Hæmomenas præcox* is sharply distinguished by the gametocytes having a peculiar form of their own. Much confusion has been caused by the belief of Grassi and Feletti, who first named these organisms with precision, that the gametocytes of *H. præcox* are a separate species, akin to *H. danilewskii*—both being placed by them in a separate genus, *Laverania*. This belief they have now abandoned ; and it therefore seems necessary also to abandon the genus *Laverania*. Several observers recognise more than one species in genus *Hæmomenas*—namely, a tertian, a quotidian, and possibly an unpigmented quotidian parasite ; but, pending further research, we have accepted only one species—*H. præcox*.

The different prevalence of the three human species at different but contiguous spots (as at Wilberforce and Tower Hill), is hardly consistent with the idea that they are merely interchangeable forms of one species of parasite.

20. Cause of Occasional Failures in Direct Cultivation.—We have noted [*paragraph 9*] that a number of *Anopheles* fed in test-tubes on cases of hæmamœbiasis, and not fertilized, failed to show parasites ; while two *Anopheles* which were fed in the same manner but which were previously fertilized, did contain them. In this case the failure may be attributed to the fact that the patients contained but few parasites and were taking quinine ; but previously one of us, in association with Dr. Daniels, obtained negative results with a considerable number of *Anopheles* fed in test-tubes on two excellent cases of crescents in Calcutta. The Italian observers also note similar failures in gnats known to be hospitable to the parasites. What is the cause ?

Grassi attributes it to immunity in certain individual gnats. This can scarcely be the

true cause, or at least the whole cause—because it sometimes happened in the Indian experiments that out of a large batch of *Culex* (according to Giles, *C. fatigans* [23]) fed on birds with *H. relicta*, every insect would become infected—or almost every insect. It is scarcely likely that the eighteen insects we refer to in paragraph 9 were all immune, while 25% of *Anopheles* of the same species caught at Wilberforce contained parasites.

The explanation which suggests itself is that something was omitted in the experiments, which is present under natural conditions, and which is essential to the cultivation of the parasites. What this is we cannot say for certain; but it is noteworthy that all the successful experiments made by one of us in India, without exception, were made with insects fed in mosquito-nets in the presence of males; while his negative experiments with various species of *Anopheles* fed on human subjects were nearly all made with females isolated in test-tubes, and therefore not fertilized.

Paragraph 16 will suggest the reason why fertilization should affect the question. The ingested blood is really required for the nutrition of the eggs. If the ova are not fertilized the blood cannot be much needed by the insect, and is possibly evacuated without some digestive process which perhaps is necessary to the vitality of the zygotes. We know that very subtle differences influence the vitality of the zygotes, because otherwise they would not perish in some species of gnats and live in others.

The point requires careful study, because until it is elucidated negative experiments cannot be entirely relied on.

The Indian experiments have shown that there is considerable variation in the number of zygotes found in gnats fed even on the same subject at the same time. This variation must depend on differences in the quantity of blood ingested by different individuals; and also possibly on some differences of quality in their digestive juices. But, at the same time, it was demonstrated that few individuals of a hospitable species entirely escape infection if fed in a natural manner.

21. Known Facts about Malaria Explained.—Caution must always be used before accepting popular theories regarding the diffusion of disease; but it is remarkable that many such ideas respecting malaria are explained and justified by the discovery that the disease is carried by *Anopheles*. We will briefly indicate the principal points.

(1.) *Malaria is endemic.*—*Anopheles* are more numerous at some spots than at others, and are entirely absent from many places.

(2.) *Swampy ground favours malaria.*—*Anopheles* breed in surface puddles.

(3.) *Level ground favours malaria.*—Pools suitable for *Anopheles* must be more common on level than on sloping ground, *e.g.*, on plains and in valleys, than on mountain sides.

(4.) *Rain favours malaria.*—*Anopheles* pools largely depend on rainfall.

(5.) *Malaria is connected with decaying rock.*—*Anopheles* may breed in hollows in rocks.

(6.) *Malaria often occurs when the soil is disturbed.*—Digging must often result in the formation of puddles suitable for *Anopheles*.

(7.) *Embankments favour malaria.*—They also favour the formation of surface puddles.

(8.) *Towns are inimical to malaria.*—Stagnant surface puddles are not likely to be as numerous in crowded, well-drained cities, as in rural areas.

(9.) *Cultivation and surface drainage tend to remove malaria.*—They also tend to remove waste water suitable for *Anopheles* and lying on the ground.

(10.) *Malaria is not particularly connected with large bodies of water.*—Gnats do not often breed in such.

(11.) *Malaria occurs on ships.*—Gnats, including *Anopheles*, visit ships.

(12.) *It is particularly dangerous to sleep in malarious places, especially at night.*—Gnats easily bite sleeping persons, and usually feed at night.

(13.) *Ground floors are most dangerous.*—Gnats seem to remain near the ground.

(14.) *Mosquito-nets protect from malaria.*—They protect also from gnats.

(15.) *Warmth favours malaria.*—It favours also gnats, and the Hæmamœbidæ within them.

In the remarkable work [1] which seems to be the first published exposition of the gnat-theory of malaria, King suggested many of these striking analogies—and indeed based his theory on them. Laveran, Manson, Bignami, and others have also independently entertained the same ideas—which may well occur to anyone who considers the subject at all. But it is now necessary to make an important correction in these views. King—and indeed other observers—seems to have thought that all gnats rise from swamps. A close student of the bionomics of gnats, however, might have almost shattered the whole argument at the outset by remarking that many of the commonest gnats do not rise from swamps at all, but from tubs, pots, etc., in the vicinity of houses; and are indeed most prevalent in the heart of large cities and in other places where there is little or no malaria. It is clear, then, that King's arguments can apply only to *certain species* of Culicidæ, namely, those which breed only in pools on the ground.

King also employs other analogies which seem to be less sound. Accepting the popular views, he considers that both malaria and gnats can be blown long distances, as to the summits of hills. It is probable that in many such cases the gnats really breed close at hand; though the observer, under the influence of the popular superstition, prefers to think that his malaria comes from the nearest marsh, although that marsh may be miles distant. King also suggests that white men are more susceptible than black men, because gnats can see the former more easily. As a matter of fact, it is always observed that gnats prefer to settle on dark surfaces; while the susceptibility of white men is more probably due to comparative absence of immunity than to more frequent infection.

22. Are other Insects besides *Anopheles* hospitable to the Human Hæmamœbidæ?—Returning to the subject just touched upon, it will be readily perceived that out of the fifteen analogies given in the last paragraph, no less than nine (the first nine) can apply only to insects which breed in pools of water on the ground. For instance, they will not apply to species of gnats which usually breed in vessels of water. Such gnats cannot be said to have a localized distribution, because they are to be found in the neighbourhood of almost every dwelling

in the tropics. They have no particular connection with swampy ground, level ground, or decaying rock. They are not particularly encouraged by rain, by digging, or by embankments. They are not removed by cultivation or surface drainage; while they swarm in cities. Hence we may almost venture to aver that if pot-breeding gnats could carry malarial fever, almost all the phenomena of the distribution of the disease would be different from what they are known to be. That is, the disease would be most prevalent in cities; would have no particular connection with marshes and level ground, or decaying rock, or rain, excavations, or embankments; and would certainly not be removable by surface drainage. In short, it would certainly be present where it is not present, and absent where it is not absent.

It will be seen from Section IV. that these considerations would seem to exculpate a large number of species of *Culex* which frequently, if not always, breed in vessels of water.

They would also exclude most other suctorial insects, such as fleas, bugs, lice, etc.

They would not, however, exclude other suctorial insects which, like *Anopheles*, breed exclusively or almost exclusively in pools of water. It is impossible, without more study, to say how many such insects exist. Certainly some species, even of *Culex*, can often breed in pools of water.

The Indian observations with "grey mosquitoes" (*Culex pipiens* type) and "brindled mosquitoes" (*Culex tæniatus* type) all proved negative with the human parasite—with the doubtful exception of one individual. The experiments were very numerous, and the feeding was done under natural conditions in mosquito nets [*paragraph 20*].

The Italian observers record negative results with Italian *Culex*.

Koch, however, thinks that *Culex* may possibly be involved [11].

On the other hand, some nine species of *Anopheles*, existing in widely separate parts of the world, have already been incriminated.

To sum up, while it seems improbable, or indeed very improbable, that the common pot-breeding species of *Culex* are involved, it is scarcely safe as yet to exculpate the genus entirely. Observations regarding other gnats and insects which breed in puddles are still required. But on the whole it appears more probable that only gnats of the genus *Anopheles* are concerned in the propagation of human malaria.

23. Are other Vertebrates besides Man hospitable to the Human Hæmamœbidæ? Dionisi records [24] that the two species of parasites found by him in bats are morphologically very similar to *H. præcox* and *H. malariæ*; and Koch says [11] that the parasite found by him in monkeys is like *H. vivax*. These parasites may perhaps be identical with the human species.

The parasites of birds can scarcely be communicable to man, being too dissimilar to the human ones. Exhaustive search in the blood of other mammals besides bats and monkeys is still required. In many places dogs are popularly supposed to get malarial fever.

The Indian experiments showed that *H. relicta* of sparrows can be conveyed by *C. fatigans* to crows and weaver-birds. Reasoning from analogy, then, we may suppose that the human

parasites may infect other animals, and the converse, through *Anopheles*; and it will be noted that monkeys and bats, in which parasites like the human ones have been found, are closely allied to man. But while no strong argument can be adduced against this view, not much can be said for it. In fact the only thing which we can find in its favour is the popular notion that malarial fever can be acquired in uninhabited localities—a notion which we will presently discuss.

24. Is the Life-History of the Hæmamœbidæ fully known?—According to the known facts, malarial fever is communicated from sick to healthy persons by the bite of *Anopheles*. But a very important question remains. Is this the only way in which the disease can be propagated; or do the parasites possess some other cycle of existence, and, perhaps, some other mode of entry into man?

Nature is so full of potentialities that we can scarcely ever venture to say absolutely, at least with regard to any of the lowest forms of life, that we know all their life-history; but when one life-cycle is known, we are not justified in believing that there may or must be another, unless there are grounds for this belief. The lower forms of life, like the higher ones, cannot have an unlimited number of life-cycles. The life-cycle of the Hæmamœbidæ as already known is complex enough—are we to suppose that they possess others? If they can live freely in air and water; if they can enter man by the respiratory or digestive tracts; even if they can pass, not only from the gnat to man, but also indefinitely from gnat to gnat, they must possess different phases adapted to these modes of existence and transference, and their whole life-history must be complex indeed. They must possess not only the known phase which adapts them for life in the gnat, but other phases which adapt them for life in the soil or water—which enable them to rise in the air and pierce the epithelium of our respiratory passages, and so on. When we consider the amount of variation in structure and function which this would entail, we can scarcely be blamed for refusing to believe in other life-cycles without good evidence.

Is there any evidence which will lead us to suppose that there is or may be another cycle? *First*, is there any evidence to be drawn from our knowledge of the disease, malarial fever? *Secondly*, is there any evidence to be drawn from our knowledge regarding the parasites themselves?

It is often stated that malarial fever exists where there are no gnats; and that persons become infected without being bitten by gnats. Were cases of this kind to be substantiated, they would certainly prove the existence of other modes of infection, either by other suctorial insects, or by some quite different route. But what value can we attach to popular statements of this kind? A perusal of paragraph 16 will suggest how easy it may be for *Anopheles* to be overlooked—especially by persons who do not make an express search for them. Moreover, when we remember that a large number of cases of fever occurring anywhere are *relapses*, and that *Anopheles* may be present only at certain seasons, we must hesitate still more in accepting such statements; while the known fact that persons can be bitten without observing it discredits the other notion. We do not say that all such statements are absolutely untrue; but we are scarcely justified in accepting them until cases have been established by competent observers.

On the other hand, it is impossible to consider the matter given in paragraph 21 without feeling that the gnat-theory suffices to explain almost all the better-established laws regarding malaria—its connection with the soil, with stagnant water, with rainfall, with rural areas, and so on. In short, there seems to be no longer *any necessity* for believing that the parasites live at all in the air or soil of infected localities. The facts on which we formerly founded our belief that they did so are now otherwise explained by the origin of *Anopheles* from surface-puddles. The facts were sound enough; our interpretation of them was wrong. Malarial fever is connected with the soil, with stagnant water, and so on, as we supposed it is; but *it is not, as we thought, the germ itself which springs from the soil—but the carrier of the germ.*

It is impossible to avoid seeing how completely this fact in the bionomics of *Anopheles* subverts the arguments in favour of the view that the pathogenetic organisms themselves have a telluric or paludal origin.

The idea that the parasites can originate in the soil, and thence pass into gnats, and from gnats to man, is opposed to all parasitological analogy. On the other hand, that they can pass alternately between men and gnats is merely an instance of a very common law among parasites.

The fact that malarial fever is communicable from the sick to the healthy has certainly been a surprising one. We thought that it is not communicable because we observed that communication does not occur outside endemic areas; while within endemic areas we attributed to an endemic poison infections which were really due to communication from the sick. By endemic (malarious) areas, we now understand simply areas which are infected by the communicating agent (*Anopheles*).

Of course the parasite itself, as well as both the hosts, must be present in an endemic area. Once present, however, there is no reason why it should not remain indefinitely so long as both the hosts remain.

So far, then, all the known facts about the prevalence of the disease seem to be easily explicable by the bionomics of the parasites and of *Anopheles*; but we now come to the consideration of the one popular theory which is not so readily explained—we mean the notion that *malarial fever may be acquired in uninhabited places.*

As a rule man lives in the society of man. Hence the vast majority of infections must take place in inhabited places—in places where previous cases of malarial fever have existed perhaps from time immemorial. Even during the most detached moments men are seldom quite solitary. Pioneers, explorers, missionaries, travellers, sportsmen, are usually accompanied by trains of servants and carriers; in order to reach uninhabited places they have to pass through native towns and villages; have to live long perhaps in native houses, and in settled camps surrounded by their followers. Ships on which malarial fever breaks out have not as a rule touched at uninhabited spots on the malarious coast, but at towns and ports where trade is carried on—and may have been visited by infected coolies, or even by infected gnats, from the shore. In all such instances, cases of fresh infection may be simply explained by communication from previously infected men—at least the possibility of this can seldom be excluded. Even in the

very rare case of complete solitaries, attacks of fever may perhaps often be attributed to relapses due to a long previous infection acquired in the haunts of men.

Travellers and sportsmen sometimes attribute their fever to having passed through a particular uninhabited but "deadly" spot. Such cases are generally questionable. On enquiry it is often found that the fever has commenced the very day, or the day after, the traveller has arrived at the place he refers to; so that the necessary phenomenon of an incubation period is wanting! A case is on record where a family of healthy natives were found living in one of the "deadliest spots" in India [25]; and we were informed that some persons who escaped from the Benin massacre, and lived several days in the reputedly deadly jungles, escaped without fever. It is certainly often possible to explain outbreaks of fever, attributed to such localities, by previous infection acquired by the suffering party in rest-houses and native villages *en route*.

If a healthy person could be carried to an uninhabited but reputedly "malarious" spot in a balloon, and should then acquire the disease, we should be forced to admit the possibility of infection in such places. Failing this, it is at least open to doubt whether autochthonous malaria does in fact exist in uninhabited localities at all.

Even assuming it does so, however, the fact would be explicable without the assistance of another life-cycle of the parasites, on the ground that *Anopheles* may possibly convey the infection from wild animals [*paragraph 23*]. For instance, both monkeys and bats are often found in large numbers in forests reputed to be malarious.

Failing even this, if it can be shown that autochthonous malaria does exist in uninhabited places, the fact would be more readily explained on the hypothesis that the Hæmamœbidæ can pass from gnat to gnat, than on any other. And this leads to the second question given above. Is there any evidence to be drawn from our knowledge regarding the parasites themselves in favour of the view that they possess another life-cycle?

The Indian observations showed that in many of the zygotes of *H. relicta* found in *C. fatigans*, certain large, black, sausage-shaped bodies occurred. These were provisionally called by one of us "black spores." They occurred not only in the zygotes, but in other parts of the gnat; facts which he at first explained on the assumption that they are produced within the zygotes, are liberated on the rupture of these, and are then distributed by the insect's circulation throughout its tissues in a manner similar to the way in which the blasts are distributed. Later, however, he observed in gnats which were not infected with Hæmamœbidæ at all, bodies which were very similar to these black spores, but which showed evidences of fission in the manner of the fission fungi, and indeed of growth entirely independent of the Hæmamœbidæ. Consequently he at once modified his statements about these bodies [21], and suggested that, though he could not say for certain what they are, they may be some parasitic organisms in gnats which have the power also of invading the zygotes of the Hæmamœbidæ. He at first thought that they might be meant for infecting the larvæ of gnats, and indeed made some negative experiments in this connection, but later abandoned all hypothesis on the subject pending further researches. Since then the "black spores" have been found by other observers, who also have failed in elucidating their nature. In Freetown we noted bodies very like them in a few *Anopheles*; but

these bodies were never seen within the zygotes, while they often occurred, mixed with what looked like segments of a fungus, within the sheath of certain muscle-fibres (*e.g.*, of the stomach), and indeed appeared to have no relation at all with the Hæmamœbidæ.

If these bodies are indeed forms of the parasites, they will certainly suggest another life-cycle. In this case they will probably infect larvæ, for the purpose of continuing the species from gnat to gnat. The probability of one of them ever reaching man's digestive or respiratory tracts must be so remote that we can scarcely imagine that they are provided for this purpose. In the meantime it remains to be proved that they have any connection with the parasites at all.

It should also be noted that many full-grown zygotes do not contain blasts,—a fact which suggests that they *may* be set apart for some other purpose. At the same time they may merely be zygotes which for some reason have not been able to ripen as early as the rest.

The method of diffusion of the disease by inoculation through the proboscis of gnats is so perfect a one that, now we are acquainted with it, it seems difficult to imagine the existence of any other—such, for instance, as the clumsy methods which in our ignorance we formerly imputed to nature. In the transference of the parasites from man to man by gnats, there is an economy which would not have existed had nature sown the organisms broadcast through the soil or air, on the chance of one of them occasionally infecting a human being.

To sum up, then—there appears at present to be no strong evidence, based on our knowledge either of the disease or of the parasites, in favour of the view that the latter possess any other life-cycle besides the one which is already known. On the other hand, the fact that malarial fever is communicated from sick to healthy persons by *Anopheles* seems sufficient to explain all the well-established laws regarding the prevalence and diffusion of the disease.

VI. PREVENTION.

25. Measures Indicated.—It is a well-known fact that malaria has been often eradicated from certain localities by cultivation and drainage of the soil. Hence we are justified in saying that the possibility of prevention has been demonstrated in some cases by experience. But cultivation and drainage are generally large and costly measures, and are often impossible. We propose now to examine whether the new observations regarding the propagation of the disease can suggest any cheaper, more precise, or more effective means of prevention.

Since the disease is certainly conveyed by *Anopheles*, it is obvious--whether this be the only mode of propagation or not--that measures taken against these insects in any locality must assuredly be at least beneficial, even if they do not result in the entire extirpation of the malady in that place.

King long ago pointed out [1] that if malarial fever be produced by gnats, prevention of the disease will consist in precautions against their bites by mosquito-nettings, window screens, and inunctions of the skin; and in measures for the exclusion or destruction of the insects, such as screens of trees, smoke, fires and lights (in which he supposed they destroy themselves), and the draining of swamps and pools. Similar ideas have occurred independently to other observers who have considered the gnat-theory. Nuttall gives [12] a summary of measures against gnats recommended by various writers such as draining or dumping the pools, or flushing them; or agitating the water by wheels turned by wind-mills; or the introduction of small fish; or by the introduction of gnat-eating birds and dragon flies; or by the use of fires, "lamp-traps," and so on, of inunctions to the skin, and of various "culicicides," such as kerosene oil; or by the plantation of water-absorbing trees. Celli and Casagrandi have recently reconsidered the subject, and have recorded a number of valuable experiments with various culicicides [26].

It will be observed that these measures can be resolved into two classes, namely :—

(1.) Precautions against the bites of gnats.

(2.) Measures for reducing their numbers.

Of course, if malarial fever be communicated only by the bite of gnats, as there is now every reason to suppose [*paragraph 24*], these measures will include the entire prophylaxis against the disease. If there be also some other method of propagation, other methods of prevention--the nature of which we cannot now indicate—will also be called for.

Note also that we refer only to the prevention of *infection*, not to the prevention of the *recurrences* of fever, which often occur in a patient years after the primary infection, and which are not connected with the original infective causes at all.

27. Operations for Reducing the Numbers of *Anopheles*.—Such operations will be divided into :—

(1.) Destruction of adults or larvæ.

(2.) Measures to prevent the insects breeding.

Where the larvæ cannot be found—as at Wilberforce—destruction of the adults can be resorted to. The gorged females, that is, the insects which are particularly liable to be dangerous, can often be killed while seated asleep in the daytime on the walls of a house. The same thing may be done on *ships*; and it is quite possible that many infections may be prevented in this manner. Native servants become very expert at the work. But, of course, such a measure will not be possible on a large scale.

The most vulnerable point in the history of gnats is when they are larvæ ; they can then be destroyed wholesale. In the case of larvæ in vessels, it suffices simply to pour out the water on the ground ; but it is more difficult to deal with larvæ in pools, drains, cisterns, wells, streams, drinking fountains, and rice-fields, etc. Even here, however, very simple measures may often suffice.

Thus small puddles can be *brushed out* with a broom, the larvæ dying on desiccation if the operation be done in dry weather. We judged that in Freetown 80 % of the puddles could be treated in this manner with little labour.

Other pools can often be *evacuated*, or *filled up with earth* by means of a few strokes of the spade. The former, however, may be impossible in perfectly level country.

Where these simple measures are impracticable recourse may be had to "*culicicides*." The most popular of these is *kerosene oil* (paraffin). We found that this can be most easily applied by means of a rag fixed on a stick. In this manner a number of puddles can be "painted" in a few minutes at the cost of but little oil ; but enough oil must be used to make a fairly permanent film. Aaron states that for five dollars sufficient oil can be purchased to make five applications to 100 acres of water [12]. Strachan recommends *olive oil* mixed with a very small proportion of *turpentine*, especially for cisterns of drinking water [27]. Nuttall quotes writers as saying that *copperas*, and even *rusty iron*, are culicicides ; certainly larvæ are not commonly found in iron vessels, except when water runs into them—as from a tap. Numerous other culicicides are suggested by the work of Celli and Casagrandi [26].

It is obvious that by these means we can destroy a large number of larvæ at any time ; and it is even possible that by repeatedly dealing with every pool in a given locality we may ultimately be able to exterminate, or almost exterminate, the insects in that locality. But there is one serious difficulty. Such operations carried out on a large scale will require constant care, involving a great deal of trouble and some expense ; while untrustworthy agents will be very likely to neglect their work. This will especially be the case if the *Anopheles* pools are very numerous. It will therefore be generally preferable to deal with the insects by *preventing them breeding* altogether.

Two ways of doing this suggest themselves—

(1.) The whole of the area of operations must be carefully dealt with by draining away or filling up every collection of water which is found to harbour *Anopheles* larvæ at any time.

(2.) Some cheap culicicide which will have the effect of rendering the pools permanently uninhabitable for the larvæ must be employed.

The first will be an engineering work, the details of which need not be considered by us. Its cost will obviously depend on the number of pools existing in the locality of operations, and on the natural difficulties in the way of draining them or filling them with earth.

The second measure will depend on the discovery of the substance which will have the effect required. It must be some cheap solid substance which kills larvæ without injuring higher animals, and which, when sprinkled on depressions in the ground, will render the pools which form there uninhabitable for the larvæ for a long time—say for months or longer. Such a substance has yet to be found by proper experiments; but we may suggest *tar* and *lime* as being possibly suitable.

When placed in water, tar constantly gives off a film like that produced by kerosene oil, but more permanent. Judging from Dr. Fielding-Ould's experiments [*Addendum II.*], it would seem to destroy larvæ in a more effective manner than oil does; but it remains to be seen how long its effects will last.

Lime is suggested by a paper by Grellet [28], of which we have seen only a review. Dr. Grellet gives instances to show that this substance is inimical to malaria; and cites particularly the example of Châtillon-sur-Loing, where malaria is said to have disappeared as the result of an extensive application of lime to the soil for agricultural purposes. As it could scarcely have acted otherwise than by rendering surface pools uninhabitable for *Anopheles* larvæ, we may surmise that lime may be a substance such as we require.

The fact that *Anopheles* do not exist in many localities where suitable pools must be present, suggests that the soil there contains constituents which are inimical to the larvæ; and it is at least probable that something like the required culicicide actually exists. If it be found, we may possess a very practicable mode of eradicating the insects from areas which we elect for operations.

In conclusion, there can be little doubt that we may often be able largely to reduce the number of *Anopheles* in some localities by these measures. If we may assume—as it seems we may—that the malariousness of a locality depends *ceteris paribus* on the number of *Anopheles* present in it, this would mean that these measures may often enable us largely to improve the health of certain places.

28. Under what Conditions do Operations against *Anopheles* promise to be most Successful?—It will, however, be at once apparent that the practicability of employing these measures in any given locality must depend on two factors, namely, (1) the number of *Anopheles* pools present, and (2) the feasibility of attacking the pools.

Judging from the careful study of the question which we made in Freetown, we

unanimously came to the conclusion that these operations, if scrupulously prosecuted, promised to be successful in that town. The pools—at least during the time when we were there, the height of the rainy season—were not very numerous; and could easily be attacked, at but little cost, by any of the measures indicated above. That is, we judged that either if the larvæ were persistently destroyed, or if the pools were carefully obliterated, the insects might be very largely reduced, if not almost exterminated, in the area studied by us [*paragraph 13, Map II.*]. It is of course impossible to speak with absolute certainty on the point until the experiment has actually been tried; but from all indications we thought this conclusion was a reasonable one.

But even if the operations ultimately prove to be successful in Freetown, it does not follow that they will be equally successful elsewhere. The conditions obtaining in Freetown do not obtain everywhere; and we have already pointed out that, owing to the hilly nature of Freetown, the pools there are both limited in number and are easy to drain [*paragraph 13*]. On the other hand, the large rainfall in Freetown (163 inches) is favourable to the formation and permanence of puddles in that place.

Judging from our observations, the number of *Anopheles* puddles in any given locality at any one time is likely to depend upon a number of factors, namely, (1) amount and continuity of rainfall, (2) humidity of the atmosphere, (3) height of the subsoil water, (4) flatness of the ground, (5) porosity of the soil, (6) presence of small fish in surface puddles, (7) use of wells and of pools for agriculture, (8) temperature; and also probably on (9) the chemical nature of the soil, and (10) presence of some vegetable food necessary to the larvæ or even to the adults. Hence we may expect much variation in the number of *Anopheles* pools in various localities.

In some tropical countries, as in parts of Assam for instance, vast level areas are almost under water during the rains. In such places the larvæ may abound everywhere; unless it happens and the point requires study—that small fish are too plentiful for them. Here it will be impossible to drain or fill up the pools.

Again, in Lagos, Strachan [27] and Fielding-Ould [*Addendum II.*] report that the pools are extremely numerous, while the ground is so flat that it will be difficult to drain them.

In Freetown the pools are much less numerous than in Lagos, and we think it possible to attack them with success.

In Accra, Fielding-Ould did not succeed in finding a single *Anopheles* pool when he was there.

In Secunderabad, in India, only one pool was detected in a considerable area examined by one of us—though others might have been overlooked.

In the Bolan Pass malaria certainly exists, though the country approximates to a desert. In such places pools must be very isolated.

In Travancore, James reports [29] that the larvæ are found in pools used for obtaining drinking-water, and also in the submerged plots of ground in which rice is grown (paddy-fields).

Possibly the larvæ may breed in *wells*—which are often very numerous in tropical cities.*

* Since this was written, Cornwall has described finding *Anopheles* larvæ in unused wells in Madras city [30].

Lastly, in many places where there is much forest or undergrowth, it may be impossible to indicate the pools at all.

Even in localities where the pools are sufficiently few, it may be difficult to deal with them. This will be especially the case with wells, pools of drinking-water, small streams, rice-fields, irrigation pools, and pools on water-logged ground.

On the other hand, operations may be very much facilitated if we can succeed in finding a cheap substance, such as was alluded to in the preceding paragraph, which will prevent the insects breeding in the pools. Suppose, for instance, that a ton of such a substance will suffice to keep a square mile of land free from *Anopheles* for a whole rainy season, we may hope to eradicate malaria from our principal towns at but little cost.

Again, as suggested in paragraph 17, if *Anopheles* live from one rainy season to another only by continuing to breed in the few pools which remain during the dry season, we may then be able to attack them with comparatively little trouble at that season. This is an indication which requires following up. It is difficult to understand how the insects tide over the dry season in places in which almost every inch of soil is then subjected to a heat sufficient, one would think, to destroy even the eggs. Unless the adults "hibernate," we may suppose that the larvæ still live in wells, or gardens, or in pools in the bed of watercourses which have nearly dried up. If this be the case, operations against the insects may be much facilitated.

On reviewing these observations, we shall see that—

(1.) Operations against *Anopheles* are least likely to be effectual in level, water-logged localities, and in places where the insects breed in pools which cannot easily be found, or cannot easily be treated.

(2.) Operations will probably be easier in country which is not quite level, or where the rainfall is not great.

(3.) They promise to be very easy in extremely dry places.

Lastly, it goes without saying that we can scarcely ever expect to deal with *Anopheles* in large rural areas. On the other hand, we may reasonably hope to reduce them, if not to exterminate them, in the principal centres of population and civilization—that is, in just the places where prevention of malaria would be most useful—provided always that we make intelligent and persistent efforts to do so.

29. Recommendations.—The method of dealing with malaria by operating against *Anopheles* would seem to have this advantage over the old method by drainage of the soil—it promises to be much cheaper. Whereas by the old method we were obliged to drain the whole of an infected area—often at great cost—we shall now be called upon to drain, or otherwise deal with, only those spots which are the source from which the insects (and, by hypothesis, the disease) emanate.

The fact that drainage has often actually banished malaria now only suggests that drainage has often actually banished *Anopheles*. By reducing this long-known method to one of greater precision, we may now reasonably hope to obtain the same effect with greater ease.

Financial considerations have frequently prohibited the employment of the old method; they can scarcely prohibit the employment of the new one—at least in many localities.

Although the new method has not yet been tested by actual experiment, all the scientific indications support the view that it will be a valid one.

The existence of the disease is in many localities such a serious source of expense, not to mention loss of trade, prosperity and life, that the small sums required for the prosecution of the new method cannot justifiably be withheld, even though it is still in the experimental stage.

Hence we feel justified in recommending that operations against *Anopheles* be included among the sanitary measures of local governments and municipalities.

We also recommend that further investigations regarding the prevention of malarial fever be prosecuted with the energy which our national obligations demand.

VII. REMARKS.

30. Miscellaneous Remarks.—The following notes may be of interest.

(1.) *Destruction of gnats, generally.*—It is a most astonishing fact that though gnats constitute a serious pest in nearly all tropical towns, one seldom hears of any efforts being made to control their numbers. Even Europeans appear to be generally quite ignorant of their source, and often allow them to breed in large numbers in tubs and flower-pots just outside their windows; and then complain of their presence and attribute it to the "locality." It would be a benefit to the community if medical men would exert their influence with local municipalities in favour of some action against these insects—not only *Anopheles*, but *Culex* as well. A single intelligent native agent, whose services could generally be obtained for one or two pounds a month, would be able to do a great deal of good in many towns, simply by going from house to house and emptying the vessels of stagnant water, or by treating them with culicicides, or by showing the occupants what to do. Sanitary inspectors might also be instructed to attend to the matter. The policy of indifference adopted at present is ridiculous.

(2.) *Houses of Europeans in the Tropics.*—We have already referred to the fact that punkahs and mosquito-nets are not as generally used in Sierra Leone as in India. In the latter country Englishmen seem to have learnt by long experience how best to live in the tropics. Not only do they employ these comforts wherever possible, but their houses are as a rule specially adapted for tropical life—they are generally commodious, well-built, and surrounded by a large open area or "compound." Undoubtedly this must assist in preserving the occupants from a great deal of fever. Gnats do not like airy, well-lighted rooms; while, if there is a large compound, infection cannot be as easily carried from adjacent houses. Moreover, in India there is generally a separate European quarter, which is as a rule built on the most elevated site present. In Freetown none of these precautions have been adopted. In addition to the neglect of punkahs and mosquito-nets, the houses are small, crowded together, mixed with the houses of the townspeople, and built in the lowest part of the town. *Anopheles* easily live in them, can bring in the infection from numerous neighbouring dwellings, and can feed uncontrolled on the occupants; while they find numerous pools suitable for them in the ditches by the side of the streets.

On the ridge behind Kortright Hill there is a large plateau over 1000 feet above sea-level, and capable of holding the houses of all the Europeans in the colony.

Serious attention ought to be given to this question of the houses of Europeans in the tropics. If *employés* are sent at all to dangerous climates, it is the manifest duty of their employers —whether these employers be the Government or private persons—to see that they are housed in

a manner most likely to preserve their health. If the nation wishes to maintain colonies in intensely malarious localities, the least it can do is to protect its servants as much as possible from the disease.

(3.) *Surface-drainage of Freetown.*—This is undoubtedly bad as a rule. The substitution of simple masonry gutters for the existing roadside ditches would probably have a considerable effect on the malaria. In some spots, however, it will be necessary to avert drainage from the hills.

(4.) *Disposal of night-soil.*—As already mentioned, a system of pit-latrines is in use in Freetown. Not only does there seem to be little dysentery or enteric fever present in the city, but the disgusting odours so frequent in Indian towns (where removal systems are adopted) are comparatively infrequent. In short, it is open to question whether this pit-system—which we have compared to a septic-tank system—is not preferable, if it can be adopted, to the ordinary Indian interception method. A pipe water-supply must of course exist at the same time ; and the subsoil water must be low.

(5.) *Filariæ in* ANOPHELES COSTALIS.—In many of the *Anopheles* caught in the Wilberforce Barracks we found *Filariæ*. All the stages described by Manson, up to the final motile stage, were present. We add photographs of an earlier and a later stage—the former from a specimen considerably distorted by the preservative (formalin).

7.—*Filaria* in *Anopheles costalis*. Young stage.

8.—*Filaria* in *Anopheles costalis*. Older stage.

It is impossible to doubt that these parasites, like the Hæmamœbidæ in the same insects, were derived from the soldiers ; but unfortunately we were unable to obtain the blood of the latter at night for examination. One of us had previously observed *F. nocturna* in the tissues of *Anopheles* fed on a patient containing the parasites [5].

(6.) *Tumba-fly and Tsetse-fly.*—We found these insects in Freetown. Accounts of them will probably be published elsewhere.

(7.) Towards the end of our stay in Freetown, one of us was attacked with fever : *H. præcox* being at once found in his blood. From the first there was no rigor. The fever was continuous, but disappeared (together with the parasites) on the fifth day, as the result of treatment with quinine. The drug was continued for six weeks, and no relapse has occurred.

(8.) *Distribution of* ANOPHELES COSTALIS.—It is always difficult, without elaborate study of perfect specimens, to come to sound conclusions respecting the species of gnats. So far, however, as we could judge from specimens obtained from various parts of the West Coast, *Anopheles costalis* appears to have a wide distribution there. See, for instance, Strachan [27] and Fielding-Ould [*Addendum II.*]. We may even conjecture—pending more certain information—that it is the principal agent of the deadly West Coast fever.

(9.) *Use of the microscope for clinical purposes.*— We beg earnestly to recommend that Laveran's discovery, which has now been made for twenty years, be used in medical practice in malarious localities. The microscope is called for, not only for diagnosis and treatment, but in order to obtain exact information regarding the nature of the various fevers prevalent in warm countries—fevers which often cannot be differentiated by the symptoms alone. For example, we do not yet know the nature of black-water fever—a point which would probably have been decided long ago if medical men had more generally adopted microscopical methods in their practice.

(10.) In conclusion, it should be strongly emphasized that many substances which have not the power actually of killing *Anopheles* larvæ may yet be capable of rendering depressions in the ground uninhabitable for them for long periods. Common salt may be effective ; but the range of substances for experiment in this connection is very large. It is to be hoped that such experiments will soon be made.

FINIS.

ADDENDUM I.

Description of Two Species of ANOPHELES *from West Africa.*
By Major G. M. Giles, M.B., F.R.C.S., I.M.S.

Owing to the sudden departure of Mr. Austen, late of the Liverpool Malaria Expedition, for the seat of war, I have been asked to draw up a formal description of the two species of *Anopheles* brought by the party from the West Coast of Africa. I should however mention that, having run over the specimens with Mr. Austen, I am indebted to him for many of the points specified, so that the description might almost be said to be a joint production.

We are agreed in considering one of the two species as undoubtedly identical with *Anopheles costalis*, Loew, originally described by him from Caffraria, but which now appears to have a wide distribution over, at any rate, a large proportion of the tropical and subtropical African littoral. The other species does not appear to have been previously described, and, at Mr. Austen's suggestion, has been named *Anopheles funestus sp. n.*

The following are the descriptions of the two species in question :—

Anopheles costalis, Loew.—Wings with the costa interrupted by patches of darker and lighter colouration, but not generally dark ; the costa has the basal third dark with two minute interruptions followed by three other dark spots, the first of which is the largest, separated by smaller yellowish interruptions ; portions of the other veins are black-scaled, but the lighter tinted scales preponderate. Abdomen not distinctly banded, but with the hind border of the segments rather darker, especially in the male. Tarsi with minute rings, mainly apical, but involving also the base of the next joint.

Description from Loew, *Berlin Ent. Zeits*, 1866, *p.* 55.

" ♀ Pale tinted, with the palpi black with white rings ; wings with uniformly black costal spots. Length of the body, 2 lines ; of the wings, $2^1/_{12}$ — $2^1/_6$ lines.

Clay coloured. The two first joints of the antennæ yellow ; remaining joints brownish. Palpi black, with a white ring on each of their joints. Thorax with a bright brownish longitudinal stripe on either side, and a distinct brownish line in the middle. Pleuræ striped and speckled with pale brown. The hairs on the thorax, scutellem, and abdomen entirely light yellowish. Legs yellowish brown ; the femora yellowish at the base ; all the outermost points of the knees and tibiæ of a yellowish colouration. Wings limpid, with pale yellowish, almost white-haired veins, with here and there patches of black hairs, so as to produce a characteristic marking of the wing ; of these spots the most striking are four placed on the anterior border of the wing and not extending beyond the first longitudinal vein, and forming elongated black spots which alternate with clearer portions of the wing-border ; the spots on the remaining part of the wing are not so distinct, because, owing to the veins being placed further apart, they nowhere approach each other sufficiently close to admit of their black portions combining to form a noticeable spot. Habitat—Caffraria, South Africa."

In the examples collected on the West Coast, with the exception of the wings, the insect is a dark brownish grey rather than clay coloured. The palpi of the ♀ have the apex broadly white, and there are, in addition, two narrow white bands on the articulations: further, their scaly covering is rougher than in *An. funestus*. The legs are dark brown, somewhat speckled with yellow, especially beneath, but show nothing of the nature of a band, except on the tarsi. In the fore legs, these have the first three joints with apical bands, which involve somewhat the contiguous bases of the next joints: in the middle legs the first three; and in the hind, the first four joints have purely apical, narrow yellowish bands. The wings are lighter than in *An. funestus*, but have a larger proportion of dark scales than in *An. rossii*, *mihi*, or, to judge from his figures, than Ficalbi's *pseudopictus* and *superpictus*. The ♂ has the terminal joint of the palpi with a dense tuft of hairs, which are brownish yellow internally and blackish externally: the only other ornament being a very minute ring on the articulation between the second and third joints.

Mr. Austen further makes the following note :—Although this species much resembles *An. pictus*, Loew (described from two females from the coast of Asia Minor, opposite the island of Rhodes) in having the front femora thickened towards the base, I think it is undoubtedly distinct. In the first place, it is much smaller (only $2\frac{1}{4}$ instead of 3 German lines). Then the two last joints of the palpi have an adornment of yellowish white scales instead of being brown; and the wings have four dark blotches on the costal margin in place of three, and all are separate, whereas in Loew's species the first and second patches are joined together on the costa itself. Lastly, the legs differ, the tarsi being banded in *costalis*.

This species forms one of a group of closely allied species, including *An. pictus*, Loew, *An. superpictus*, Grassi, *An. rossii*, *mihi*, *An. vanus*, Walker, and some others, which agree in having the wings with the costal margin adorned with alternate dark and light spots, while the wing as a whole is not dark tinted. When closely examined, however, the details of the adornment of the wings, as well as that of the palpi and other appendages, afford sufficient points of constant difference to assure us that they are specifically distinct.

The other (and new) species is much more darkly tinted, the white interruptions on the costa and other veins being so much smaller than the dark portions that it is fairer to call their wings black, with white spots rather than the reverse. In this respect it forms a link between the paler forms and the densely black-scaled wings of two species, also new, but as yet undescribed, which were brought back from India by Major Ross, and are now in the British Museum.

Anopheles funestus, sp. n.—Wings with the costa marked by interrupted darker and lighter colouration, the dark portions preponderating; on the actual costa there are five interruptions, the two basal being connected by dark scales on the auxillary vein; there are also white areas over the transverse veins, the stem of the posterior fork-cell, and the bases of the 5th and 6th longitudinal veins. Abdomen unbanded, black. Tarsi uniformly black.

♀ General colouration black, with but little adornment. Head black, with a crest of scattered white scales; eyes with a minute white margin; proboscis black, a little paler at the apex; antennæ black, with scanty whitish verticils; palpi black, very smooth, with the apex and two narrow bands on the articulations white. Thorax black, clothed with white scales over the greater part of the dorsum. Legs black, the only relief being a scarcely perceptible paler knee-ring. Wings ornamented as above described; the anterior fork-cell long, parallel-sided, with a short stem; the posterior, much shorter, wider, and wedge-shaped, with a longer stem; supernumerary and middle transverse veins of fair length, in one line, the posterior, shorter and more than twice its length internal to it; the scales of the internal fringe with minute white interruptions opposite the junctions of the longitudinal veins with the margin. Halteres with the knob black and the stem rather lighter. Abdomen black, glabrous, with very scattered white hairs.

In the ♂ there is no obvious banding of the palpi, but the apex of the last joint is grey, followed by a narrow black ring, and then by an even narrower pale ring, the rest of the appendage being of the same funereal tint as the rest of the body. The antennæ are black with dense black verticils, which, however, show a certain paler lustre in certain lights. The abdomen is black. The wings, though they resemble those of the ♀ closely in ornamentation, differ somewhat in venation,

the anterior fork-cell being twice as long as the posterior, and its stem much the shorter, though both stems are rather long, while the posterior is very short, and but little wider than the anterior.

Length, excluding the proboscis, 2.6 mm.

Habitat.—Freetown, West Coast of Africa.

Owing to the absence of ornamentation, the description of this species is necessarily brief, but it will, I trust, suffice to distinguish it from any neighbouring species.

G. M. GILES, M.B., F.R.C.S., Major I.M.S.

ADDENDUM II.

Observations at Freetown, Accra, and Lagos.
By R. Fielding-Ould, M.A., M.B.

I. SIERRA LEONE.

In accordance with instructions I joined Major Ross at Sierra Leone in September last, and during the remainder of his stay there co-operated with him in his researches; and later, when he returned to England, I continued the work of the expedition in Freetown.

The habitat and "breeding puddles" of the *Anopheles* which had been observed infesting the Wilberforce barracks we had been unable to discover, and I determined to make further and more rigorous search through the surrounding country than had been possible hitherto. In this I received the valuable assistance of Captain Smith, R.A.M.C.; but, notwithstanding the most careful explorations, we were unable to find any *Anopheles*-bearing puddles nearer to the barracks than 1,500 yards. Those we found were more or less permanent pools in the crevices of rocks below Signal Hill; and some others were found in Murray Town. I am inclined to think that these puddles are the puddles that supply the mosquitoes which infest the barracks. It has been observed that the *Anopheles* are only to be found in the barracks intermittently—that is to say, a swarm may be present for two or three days, and then for a week no mosquitoes are to be seen. This fact, and the impossibility of finding any puddles nearer than a mile, I venture to explain by the hypothesis that the wind is an important factor in the matter. The prevailing wind blows over the puddles that I have mentioned in the direction of the barracks, and by its means, when favourable, the mosquitoes may be able to travel a long distance—quite contrary to their ordinary habits.

In support of this view it may be mentioned that many facts are on record which seem to indicate that the poison may be carried to some distance by winds. Again, it has been noticed that of the two banks of a stream in a malarious district, that towards which the prevailing winds blow is often the most affected.

During the remainder of my stay in Freetown I was kept supplied by consignments of mosquitoes from the barracks, and I also obtained some from the houses of natives known to be suffering from fever. Dissection of these gave substantially the same results obtained by Major Ross. I include with these notes some drawings, made on the spot, of microscopical preparations, which show various phases of the malarial parasite in the gnat (Plate IV.)

Out of 29 *Anopheles* examined by me, six, or 18 per cent., gave definite parasites; while 17 *Culex* gave in every case negative results.

I made experiments on the puddles at Freetown with both tar and kerosene as culicicides. Both these proved effectual as larva-destroyers, but the former is, in my opinion, likely to prove the

more successful ultimately. In the same street many puddles bearing larvæ were treated—some with kerosene and some with tar. After 24 hours all were free from larvæ. After a further interval the puddles were again examined ; with the result that all the puddles treated with tar were still free from larvæ, while many of those treated with kerosene contained larvæ. This result is due to the greater evanescence of the kerosene film.

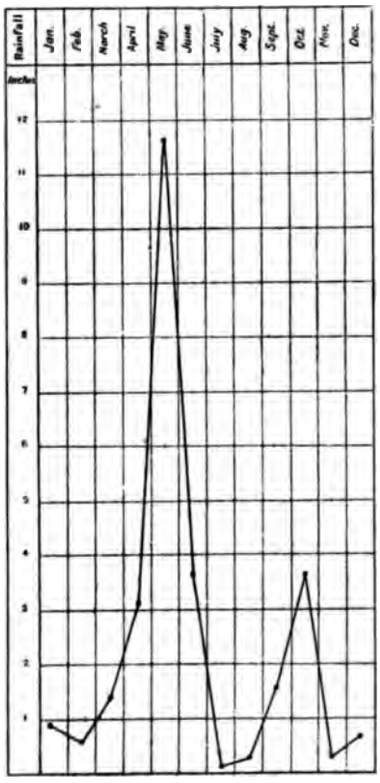
II. ACCRA.

On October 18th I landed at Accra, and after being very kindly received by the Governor of the Gold Coast, who spontaneously offered me all the assistance in his power, I was fortunate enough to meet Dr. Chalmers, who kindly offered me quarters, and for whose hospitality and assistance in my work I cannot too warmly express my gratitude.

Accra, a straggling town consisting roughly of four parts—native, commercial, official, and Christiansborg—(*vide* Map III.) is situated on a perfectly flat plateau on the coast, about 25 feet above sea level. From the level of the town to the sea-shore stretch broken and crumbling rocks ; and on the other side, northward of the town, the country is absolutely flat (save for a slight depression here and there) for 26 miles, until the Aburi Hills are reached.

During my stay at Accra I was unable to find any puddles, and consequently *Anopheles*, notwithstanding a rigorous search throughout the entire district. To those who know the conditions under which *Anopheles* were found at Sierra Leone this will not be surprising, for the conditions at Accra are totally different ; the rainfall never exceeds 27 inches per annum, as compared with 170 inches at Sierra Leone. Again, at Sierra Leone the geological formation is volcanic rock, whereas Accra is built on sand and gravel ; consequently the small rainfall is very rapidly absorbed by the porous ground. There are no trees, and therefore there is no shade.

RAINFALL AT ACCRA, 1897



I include here a chart showing the curve of the rainfall at Accra.

It has been found that the number of fever cases is greatest during the second quarter of the year, that is to say, just before, during, and just after the period of greatest rainfall (*vide* chart). Another rise in the number of cases occurs in the last quarter ; when again it will be noticed that the rainfall increases. At this time too the "*Harmattan*" blows, and there is a great fall in the temperature at nightfall. The increased liability to chill at this time no doubt, to some extent, explains the increase in the number of fever cases. I may say that during 1899 the rainfall has been somewhat less than usual.

Not only was I unable to find any specimens of *Anopheles* in Accra, but Dr. Chalmers, an enthusiastic entomologist, also had not seen any during the three months prior to my arrival. Other kinds of mosquitoes were present without limit ; and I seized the opportunity to examine as many of the *Culex* species as possible. Fifty-two specimens I dissected, but in none did I find zygotes. Before deciding that *Anopheles* is never found at Accra, we must wait to see if they are present when the rains are at their height, that is to say, in May or June.

These being the chief facts which I obtained as regards the mosquitoes of Accra, one would

naturally expect to find that malarial infection was comparatively a rare event there. And this is precisely what we do find. All my enquiries from local medical men and colonial surgeons led me to believe that there had been no case of "original infection" for two and a half months before I arrived. During this time there had of course been "recurrent cases," though these had not been numerous—only thirteen among Europeans. These, I venture to say, are important points; but I do not wish to lay too much stress on them, for I fully realize that it would be rash to draw too rigid conclusions from observations carried on over so limited a time; but it is, in conjunction with facts observed elsewhere, at least significant of the influence of the mosquito in the propagation of malaria.

It should be noted in this connection that all authorities, in each of the three Colonies that I had the opportunity of visiting, agreed that 1899 had been an exceptionally healthy year; so that probably the number of fever cases was below the average.

Thanks to the efforts of the present Governor—Sir Frederick Hodgeson—the official population is now better housed: spacious and well-ventilated bungalows have been provided, and these have been removed nearly a mile eastward, so that the native town with its squalor and overcrowding is no longer the menace that it was (*vide* Map III.). On the other hand, many of the habitations of the commercial population are badly built and badly ventilated; some are still in close proximity to the native huts, and some to that part of the sea-shore which I shall refer to again as practically a public latrine. To improve the sanitary condition of the native town much has recently been done in some districts. A fire in 1896 burnt down many of the huts in one part, and afforded an opportunity of opening up the maze of squalor that previously existed. Broad streets were laid down, a partial system of drainage started, a stinking pool in the market place filled up and better houses built—with the result that disease of all kinds in this district diminished. Several districts remain in all their primitiveness, and similar treatment could not fail to bring about a marked improvement. But the Government, though alive to its responsibilities in this matter, are handicapped by a want of money for the purpose, and also not a little by restrictions imposed at home by a mistaken and sentimental regard for what are called "native rights." Already so much has been done with good results that, in my opinion, advanced with the greatest diffidence, Accra is not the death-trap its reputation would warrant one to believe. This reputation has been built up partly by the many cases of sickness imported from the hinterland; and to get a right conception of the general health of Accra it is necessary to draw a distinct line between the seat of government and the northern territories of the colony. I do not mean to say that it will ever become a health resort, nor do I think it would be wise to extend (as has been by some suggested, including the late Governor, Sir William Maxwell) the length of the "tour" of service. The climate, quite apart from any specific disease which may there exist, has a definite reducing and depressing effect on European constitutions. However healthy a man may be on his arrival on the Gold Coast, sooner or later he becomes anæmic or dyspeptic, and often his nervous system is so affected that a condition of extreme irritability is induced; insomnia often follows, and he becomes totally unfit for work.

It should not be overlooked that the difficulty—often impossibility—of procuring fresh meat and vegetables is after a time a serious tax on any constitution, however strong. There is a widespread belief that alcoholism is rampant on the West Coast; I may say here that, though I had good opportunities of judging, I did not find it so in any of the three Colonies I visited.

That part of Accra known as the "native town" (*vide* Map III.) needs further consideration here, in order to make it clear that what I have already said may not seem exaggerated, and that it is in fact a standing menace to the health of the community at large.

In this part of the town there are no streets. Winding alleys, unpaved, and so narrow that two persons cannot stand abreast, do duty for streets. These alleys have interminable ramifications, and form so complex a maze that it was found impossible by the surveying authorities to chart them. Numberless naked children disport themselves in these gutters, and many mangy dogs are seen wandering about under no control. In addition to these, sheep and goats of a more or less unhealthy appearance roam the byeways. Filth of all sorts is found lying broadcast in the alleys; stinking garbage, decaying vegetables,

etc.; and, to crown all, the natives wash themselves openly in the puddles they have made for the purpose. The children for the most part use these byeways as latrines. Mosquitoes abound, and the odour is often frightful. The huts are made of mud roofed in with leaves. They are overcrowded to an appalling extent—8 or 10 persons often occupying a hut 9 or 10 feet square. Scattered here and there through this district are groups of uninhabited, roofless and often ruined huts. These are used by the neighbouring families as places in which to discharge their refuse, and also as latrines. Every now and then one comes across a butt or other receptacle filled with stinking water, infested with mosquitoes, and infected with bacteria. The latter I was only able to examine microscopically. The former were all of the genus *Culex* —no *Anopheles* were found. A complete absence of any system of drainage completes what to a European eye is nothing but a noisome and a pestilential district. The danger of such neighbours to the white population is obvious, and some of the commercial community, as I have said, live very close indeed to those parts of the town. This danger is, I think, increased by the fact that the prevailing wind sweeps over and through these native dens before reaching the houses of the white inhabitants. Public latrines have been indeed provided by the Government on the sea-shore, but I am of opinion that these are themselves not wholly without danger. Some are flushed by the sea; but on October 20 two were entirely dry—and that on a day when the tide was of quite average height. Much of the open sea-shore is used as a public latrine too; and it is easy to imagine in such a climate the condition of affairs thus brought about. Any infective material deposited here is liable also to be carried by the prevailing wind straight to the European quarters. The problem of drainage and general sanitation is no doubt a very difficult one to solve in a place which is so flat, and where there is no water supply (the inhabitants are entirely dependent on a scanty rainfall). But I venture to think much benefit would result from the running of broad streets through the congested districts of the native population, and the destruction of uninhabited houses. An attempt is made by the police to keep the streets in a clean and healthy condition; but it is impossible to exercise anything but very incomplete supervision over these unlighted rabbit-warrens. All wandering dogs and other animals not properly looked after should be destroyed, for that there is a possibility of their carrying diseases I do not think anyone is inclined to deny. I am disposed to think that the sea latrines should be done away with, and earth-to-earth land latrines substituted. These latter are used with complete success in some parts of Lagos, though there too there are some latrines open to the lagoons.

The suburb of Christiansborg shown on the map has no such plague spots as I have described in the native town of Accra. Here there are broad streets, well kept, and for the most part clean; the houses are not crowded together, and there is a fairly complete system of drainage. The drains are open, wide, and made of cement. Between this suburb and Accra there are a few acres devoted to surface pools, which the natives enclose to catch the rain for various domestic uses; and on these pools they are entirely dependent for their water supply. Some contained mosquitoes (all *Culex*, no *Anopheles*), various weeds and algæ; but were sweet and clean for the most part.

It may be surprising that so near Accra a suburb with the above characteristics should exist; but it is easily explained: Christiansborg Castle was at one time the Governor's residence, and Christiansborg itself the seat of government. At this time some thousands of pounds must have been spent to produce the good result I have sketched above. The present Governor is doing everything in his power to improve sanitation in Accra, and though, as I have said, he is hampered in various ways, slowly but surely an improved state of things is showing itself.

The authorities and West Coast medical men generally are now fully awake to the fact that all has not yet been done that may be done to improve sanitation, and in the next few years I anticipate a definite improvement in this respect, and also a greater activity in scientific medical research. At present any research is carried on under almost disheartening difficulties. Instruments rust, microscopes and other lenses become cloudy (even the cover glasses and slides which I took out with me became useless), and bacteriological research is quite impossible under present conditions.

Now, however, I think I may say a new epoch is about to begin in the scientific study of diseases prevalent on the West Coast. The Government of the Gold Coast has sanctioned a preliminary

expenditure of £500 for the formation of a medical research laboratory at Accra, and before I left I had the pleasure of seeing the building almost ready and fit to receive its scientific equipment. Much, too, might be done, I venture to suggest, throughout the Colony in this direction. At present no facilities—not even microscopes—are provided for the assistant or Colonial surgeons on duty in the hinterland. When one considers that by the microscope we are alone able to diagnose malaria with certainty, this complete absence of proper instruments is not only appalling, but incomprehensible. In this connection I may say that there is reason to believe that many cases are wrongly attributed to the malaria parasite, and earnestly treated with quinine. Were it feasible, though for certain political reasons it may not be possible, I think better results would accrue if each assistant Colonial surgeon had a definite district assigned to him with a permanent headquarters, where he might, with a small outfit of scientific apparatus (at least the microscope, which he is at present without), do much good work in the interests of tropical medicine. The Colonial surgeons are at present so continuously travelling (often unavoidably, no doubt), that to expect any scientific work would be ridiculous. But I do not think that a central research laboratory at Accra—receiving films of blood, pathological specimens, etc., for examination—will entirely meet the case, especially in a climate where morbid specimens so rapidly change their character. There is already a well-appointed and well-managed hospital at Accra, open for the reception of white and black alike ; and here, too, improvements are being made.

There is no doubt that at Accra the greatest desideratum is a proper water-supply. Up to the present time, though borings in every direction have repeatedly been made, no water fit for drinking purposes has as yet been discovered. Rain-water can alone be obtained, and must be stored in tanks for long periods—during which time it runs great risk of contamination, though every care be taken. A scheme is on foot to bring water from the Aburi hills, 26 miles away, at the cost of over £100,000. But considering the great expense of this, and the great hindrance to all forms of business caused by the short term of service imposed by the climate, it is surprising that only during the last year has the possibility of moving the whole community to Aburi been mooted.

Parasites were not found in any out of 47 *Culex* examined by me at Accra.

III. LAGOS.

I proceeded to Lagos, and arrived there on October 27. The Chief Medical Officer of the Colony (Dr. Henry Strachan) offered me quarters ; and for the great hospitality he showed me during the whole of my stay I wish here to express my sincere appreciation and gratitude. Lagos is the largest town I visited. It has roughly 75,000 inhabitants, of which about 150-200 are Europeans. Any estimate of the population is necessarily only approximate, because the taking of the census is the signal for a general exodus of the natives for the bush. The town itself is built on an island surrounded by lagoons (*vide* Map IV.). The highest point is 19 feet above the level of the water, while, on the other hand, several parts are actually below sea-level. The island consists geologically of sand deposited on the top of mangrove swamps ; and borings reveal the existence of alternate strata of sand and decaying vegetable matter. The swamps (shaded on the map) are seen to almost entirely surround the island. These evil-smelling quagmires consist largely of mangrove trees and black mud—the latter has a peculiarly offensive odour, due chiefly, I think, to the evolution of sulphuretted hydrogen gas. Under the microscope I found this mud to consist chiefly of sand and decaying vegetable matter : many protozoa also were present. The Europeans live almost entirely in one limited part of the town, their quarters being stretched out along the edge of the south-western lagoon (*vide* Map IV.). They are therefore, it will be observed, surrounded on two sides by swamp and lagoon, and on the other two sides by native habitations. These latter I need not describe, as they bear a general resemblance to those existing at Accra.

The geological formation of Lagos being such as I have described, it is not surprising that puddles suitable for the breeding of *Anopheles* are plentiful. The island may almost be said to be one immense puddle ; for the ground everywhere lends itself to the formation of small collections of water and mud on

the occasion of the slightest rainfall. In this respect the contrast presented between Lagos and Accra cannot fail to be noticed. *Anopheles* I found to be so widely distributed that it was impossible for me to make any distinction in favour of one part of the town as compared with another. In every native hut—in all European quarters—in Government House—even in the hospital—I never failed to find some specimens. My search for parasites was not equally successful; for, although I dissected 37 specimens, I was only in one able to satisfy myself of the presence of zygotes.*

During my stay at Lagos there was very little fever; only two cases were admitted into hospital, and no out-patients presented themselves for treatment. The blood of both these patients I examined; but no parasites were present, though the examination was made on more than one occasion. This did not however surprise me, for both were mild cases, and had been taking large doses of quinine for six days before admission. *Anopheles* caught in the wards, and others "fed" on these same two cases, gave also negative results.

The single specimen in which I found zygotes was caught in a native (Hausa) hut; but I was unable to trace the cases, and so failed to obtain other specimens. I also, at Lagos, dissected 17 specimens of *Culex;* but, as at Accra, these invariably gave negative results. The problem of how best to deal with the *Anopheles* and their breeding-places is a peculiarly difficult one at Lagos. The peculiarities of the soil, and the extreme flatness of the island, render it impossible, in my opinion, for drainage to be an effectual solution. For similar reasons, and also on account of the universal prevalence of the mosquitoes—*Anopheles*—it does not seem to me likely that the application of kerosene or tar to the puddles will be altogether a successful method. In some few places an attempt has been made to drain marshy ground into the lagoons, and occasionally this has no doubt done good; but, taking the town as whole, this is not feasible, and some other method of extermination must be devised. The Government, if I may say so, have certainly been acting in the right direction by filling up some of the more aggressive mangrove swamps with sand; but this is necessarily a long and tedious business, and at the best, only a portion of the evil can be treated in this way.

In these swamps, as also at Sierra Leone, I looked for *Anopheles*, but in neither place did this particular genus seem to frequent them. Both drainage, as far as it is possible, and the application of kerosene would no doubt do something to improve matters; but until some further measures can be devised, the people of Lagos must rely chiefly on the general and less direct means that were recommended at Sierra Leone, *e.g.*, the intelligent use of mosquito-nets and blinds, etc.—of both of which there is at present a surprising absence.

The "Rest-House," which is used as temporary quarters for Government officials, I found swarming with *Anopheles;* and I was able to obtain particulars of several cases of malarial infection occurring there. Before my arrival, and indeed before the mosquito theory had been brought prominently forward, several officials dated their fever from nights spent at this "Rest-House" among hosts of what everyone recognised as a peculiarly vicious variety of mosquito. I am now referring to several well-authenticated cases, the details of which Dr. Strachan is, I believe, about to publish. From a consideration of these cases one is led to the conclusion that malaria has a definite incubation period of from 7 to 21 days.

I include here a chart which shows the rainfall, sub-soil water, and fever curves in Lagos for twelve months. It will be seen that these curves are as nearly as possible coincident, the maximum number of fever cases occurring a little later than the point of highest sub-soil water. It is impossible to suppose that this is a mere casual coincidence, and that the two curves have not a definite relation to one another. The bearing of these observations on the validity of the mosquito theory is obvious, for we know that the *Anopheles* are most prevalent when the sub-soil water is highest.

It has often been observed in many parts of the world that the upturnings of earth, and excavations generally, were accompanied by an increase of malaria: it is certain that the turning up of soil will aggravate the manifestations in districts where malaria is permanently endemic. This has also

* This can be attributed to the gnats not having become previously infected from patients.

been observed in West Africa, and especially by railway engineers—who perhaps suffer more from fever than any other section of the white population. I was anxious to see if this could be explained on the ground that the upturning of earth caused an increase of *Anopheles* puddles. When, therefore, the Governor, Sir William Macgregor, M.D., K.C.M.G., invited me to go by special train up the Lagos Railway from Abutemetta to Aro, I eagerly seized the opportunity. I found that the railway track was entirely a made track, and that to provide the soil for embankments, etc., many excavated pits had been made. In some places, for a mile or more these pits remained gaping at intervals of about twenty yards. In all that I examined there were puddles of varying depth and size, and without exception these puddles contained *Anopheles* larvæ. This is, to my mind, a point of great interest and importance, and may entirely explain why in West Africa, at any rate, railway engineers and officials are peculiarly and pre-eminently exposed to malarial infection.

Throughout the West Coast malaria is ever before men's eyes as an inevitable evil which all must suffer at some time or another; and the result is that any rise of temperature is immediately diagnosed as malaria, and rigorously treated with quinine. I saw at least one case in which a lady suffering from a typical attack of pneumonia (temp. 104° F.; dyspnœa, complete evidence of consolidation, etc.) was diagnosed as malaria, was treated with quinine, and was going to the bad. Fortunately a medical man, comparatively new to the coast, with a mind open and not befogged by coast tradition, was called in, and a correct diagnosis made: the lady recovered with a typical crisis.

This is a glaring instance of the tendency on the coast to diagnose any rise of temperature, be it heat insolation, pleurisy, rheumatism, or even constipation, as malaria. The absence of microscopes, and the apparent want of belief in the advantage of microscopical investigation, is responsible to a great extent for this state of affairs; but traditional ideas born before the discoveries of Laveran still hold sway, and cannot wholly be exculpated.

It is noteworthy that black-water fever occurs at all periods of the year; and, as far as I can judge, its curve of incidence bears no relation whatever to any of the three curves on the chart. Black-water fever occurs chiefly among two classes of the European community, viz.—

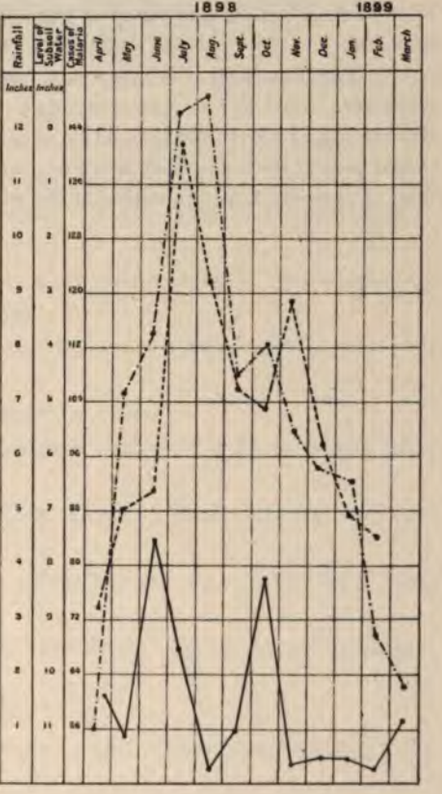
(*a*) Roman Catholic priests and nuns.

(*b*) German commercial community.

Investigation shows that the connecting link between these two classes, and the fact that distinguishes them from other classes, is that they are the most overworked, the worst fed, and, as a general rule, obtain the least leave to Europe. Some never leave Lagos.

RAINFALL, SUB-SOIL WATER AND FEVER AT LAGOS

Rainfall shown thus ——
Sub-soil Water · · · · · ·
Fever · — · — ·



I was informed that black-water fever is becoming more frequent in West Africa, but that its virulence is distinctly diminishing. As in the case of malaria, microscopical examinations of the blood in black-water fever is the exception rather than the rule, but I never met any medical man in West Africa who had been able to demonstrate blood-parasites in these cases. From a consideration of these facts I am inclined to think that black-water fever will one day prove to be a fever *sui generis*, and wholly unrelated to the malarial parasite.

In Lagos, again, as in the Gold Coast, I venture to say that the disinterested activity of the English Schools of Tropical Medicine is bearing good fruit. Before I left I heard that 15 microscopes had been

ordered for the use of the surgeons in the Colonial service. A laboratory for research is also being built, and cannot fail to do good work. In the hinterland, too, facilities for microscopical investigation are being provided.

There remains the water supply of Lagos to be mentioned. The absence of a proper water supply, as in Accra, must necessarily bear its share of responsibility for much of the unhealthiness that obtains. In Lagos, again, the people are dependent on the rainfall alone for their supply of water.

The rainfall in Lagos amounts on an average to 70 inches per annum, and must be stored in tanks for use during long periods. The risk of contamination is great, and until lately no great effort was made to keep the tanks in good order. Recently, in consequence of the recommendations of the C.M.O., a better state of things has been inaugurated, and every effort is now being made to keep the tanks clean and free from vegetable growth and mosquito larvæ. Public latrines are provided by the Government. Some are open to the lagoons; others, the most sanitary I think, are earth latrines; and it would be well if more of the latter were provided, and those open to the lagoons closed.

I should have mentioned that there are in places shallow surface wells from which the *natives* obtain most of their water: into many of them the sub-soil water drains, and their use can only be viewed with suspicion.

The hinterland is in many parts healthy, many districts affording high lands and good water, where white men could live in perfect security. It was suggested by the late governor, Sir William Macallum, that the seat of government should be removed some twelve miles up the railway from Abutemetta. This should present no insuperable difficulties, and would be an untold benefit to the Government officials, who form at present a large proportion of the white population of Lagos.

REFERENCES.

1. KING.—Mosquitoes and malaria. Popular Science Monthly, Sept., 1883.
2. LAVERAN.—Traité des fièvres palustres. Paris, 1884.
3. MANSON.—On the nature and significance of the crescentic and flagellate bodies in malarial blood. Brit. Med. Journ., 1894, Vol. II.
4. „ On the life-history of the malaria germ outside the human body. Brit. Med. Journ., 1896, Vol. I.
5. Ross.—Pigmented cells in mosquitoes. Brit. Med. Journ., 1897, Vol. II. ; and *ib.*, 1898, Vol. I.
6. „ Report on the cultivation of *Proteosoma* Labbé in grey mosquitoes, dated 21st May. Government Printing, India, Calcutta. Also Ind. Med. Gaz., 1898.
7. „ Preliminary report on the infection of birds, etc., dated 11th Oct., 1898. Government Printing, India, 1898. Also Ind. Med. Gaz., 1899.
8. DANIELS.—Transmission of *Proteosoma* to birds by the mosquito. Proc. Royal Society, Vol. LXIV.
9. MACCALLUM.—On the flagellate form of the malarial parasite. Lancet, Vol. II., 1897.
10. GRASSI, BIGNAMI, BASTIANELLI.—Numerous papers. Rendiconti della R. Accad. dei Lincei, 1898 and 1899. Also, Annali d'Igiene Sperimentale, 1899.
11. KOCH.—Ueber der Entwickelung der Malariaparasiten. Zeitschrift fûr Hygiene, etc., 1899.
12. NUTTALL.—Neuere Untersuchungen über Malaria, etc. Hygienische Rundschau, 1898, 1899. Also, On the rôle of insects...on the spread of diseases. Johns Hopkins Hospital Reports, Vol. VIII., Baltimore.
13. Ross.—Report to Director-General Indian Medical Service on the extermination of mosquitoes, dated 16th February, 1899. *See* Ind. Med. Gaz., 1899.
14. „ On the possibility of extirpating malaria from certain localities by a new method. Brit. Med. Journ., July 1st, 1899.
15. „ Life-history of the parasites of malaria. Nature, August 3rd, 1899.
16. WILSON.—Notes on malaria in connection with meteorological conditions at Sierra Leone, 1899. H. K. Lewis, London.
17. DUGGAN.—The parasite of malaria in the fevers of Sierra Leone. Medico-Chirurgical Trans., Vol. LXXX., 1897.
18. BANCROFT.—On the metamorphosis of the young form of *Filaria bancrofti*, etc. Journ. Trop. Med., Nov., 1899.
19. GRASSI and DIONISI.—Il ciclo evolutivo degli emosporidi. Rendiconti della R. Accad. dei Lincei, 1898.
20. MESNIL.—Coccidies et paludisme. Revue Gén. des Sciences, 1899.
21. Ross.—Report on the nature of kala-azar, dated 13th January, 1899. Government Printing, India, Calcutta.
22. Report of the Liverpool School of Tropical Diseases, 1899.
23. GILES.—A description of the Culicidæ employed by Major R. Ross. Journ. Trop. Med., 1899.
24. DIONISI.—I parassiti endoglobulari dei pipistrelli. Rendiconti della R. Accad. dei Lincei, 1898.
25. Ross.—Report on a preliminary investigation into malaria in the Sigur Ghat. Ind. Med. Gaz., 1898.
26. CELLI and CASAGRANDI.—Per la distruzione delle zanzare. Ann. d'Igiene Sperimentali, 1899.
27. STRACHAN.—Notes from Lagos, West Africa. Journ. Trop. Med., 1899.
28. GRELLET.—Revue Scientifique, Oct. 21st, 1899, p. 530.
29. JAMES.—The collection of mosquitoes, etc. Ind. Med. Gaz., 1899.
30. CORNWALL.—The extermination of malaria. Ind. Med. Gaz., Jan., 1900.

DESCRIPTION OF THE PLATES.

NOTE.—Plates I., II., and III. consist of photographs taken mostly from unstained preparations preserved in formalin. Formalin accentuates the outline of the zygotes; on the other hand, a small amount of detail has been lost in the collotype process. Hence the result is that the photographs show the parasites almost exactly as they are seen in fresh preparations of the tissues of the gnat, except that the granules of melanin do not come out with sufficient clearness in all the figures. None of the photographs have been in any way retouched by hand.

PLATE I.

Figure 1 (× 500).—Very small zygotes of *Hæmamœba relicta* attached to stomach wall of *Culex fatigans*. The melanin has become enlarged by the formalin. Coloured.

Figure 2 (× 500).—Very small zygotes of *H. relicta* attached to stomach wall of C. fatigans. Unstained. The melanin is of the natural size.

Figure 3 (× 500).—Very small zygotes of *H. vivax* attached to stomach wall of *Anopheles costalis*. Unstained. Melanin scarcely appreciable in the collotype.

Figure 4 (× 500).—Very small zygotes of H. malariæ in *A. funestus*. Unstained.

Figure 5 (× 500).—Small zygotes of *Hæmomenas præcox* in *A. costalis*. Unstained.

Figure 6 (× 500).—Small zygotes of *H. malariæ* in *A. costalis*. Unstained.

PLATE II.

Figure 7 (× 500).—Larger zygotes of *H. relicta* in *C. fatigans*. One well shown protruding into the body cavity of the host. Unstained.

Figure 8 (× 500).—Still larger zygotes of *H. vivax* in A. costalis, showing great delicacy of the outline. Unstained.

Figure 9 (× 500).—Medium sized zygotes of one of the human species in *A. costalis*. The focal plane is taken through one of the blastophores (in the middle of the zygote). The blasts are already nearly mature. Unstained.

Figure 10 (× 500).—A zygote of *H. relicta* about two-thirds of the full size, protruding into body cavity, and containing blasts which are already nearly mature. Unstained.

Figure 11 (× 500).—A full-sized zygote of *H. relicta* full of mature blasts, many of which are seen to be packed side by side (near the lower margin). Unstained.

Figure 12 (× about 60).—Stomach of *C. fatigans* with mature zygotes of *H. relicta*, as seen by a low power. Coloured.

PLATE III.

Figure 13 (× 500).—Mature zygote of *H. relicta* full of blasts; and also capsule of another (to the left) containing "black spores." Unstained.

Figure 14 (× about 400).—Blasts and "black spores" of *H. relicta* in *C. fatigans*, apparently ejected together from the capsule of a zygote. Unstained. The "black spores" do not seem to be mature.

Figure 15 (× 400).—Blasts of *H. relicta* lying free in the juices of the thorax of *C. fatigans*. Unstained. Many lie in perspective.

Figure 16 (× 500).—Four blasts of *H. relicta* in juices of C. fatigans, dried and stained.

Figure 17 (× 500).—Blasts of *H. relicta*, dried but not stained.

Figure 18 (× about 400).—Blasts of one of the human Hæmamœbidæ in the salivary gland of *A. costalis*. Unstained. The blasts are closely packed within the salivary cells, and also lie outside the cells, within the capsule.

PLATE IV.

Drawings, by Dr. Fielding-Ould, of the Hæmamœbidæ found by him in *Anopheles* in Sierra Leone.

Figures 1-9.—Zygotes—apparently of *H. vivax*.

Figure 10.—A zygote discharging blasts.

Figure 11.—Individual blasts.

PLATE V.

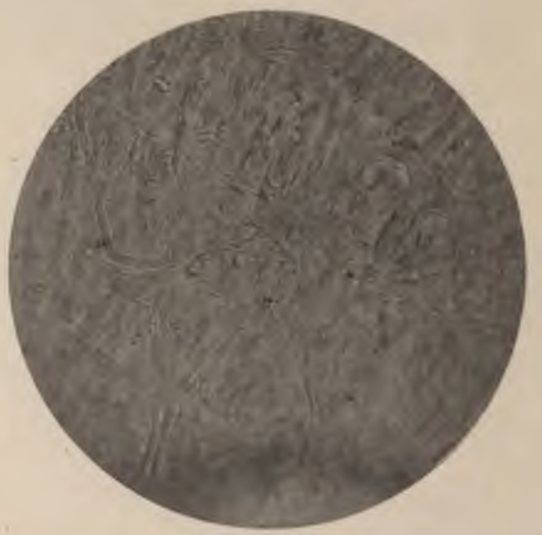
Drawings of *Anopheles costalis* and *Anopheles funestus*. Made at the British Museum.

PLATE I.

1.

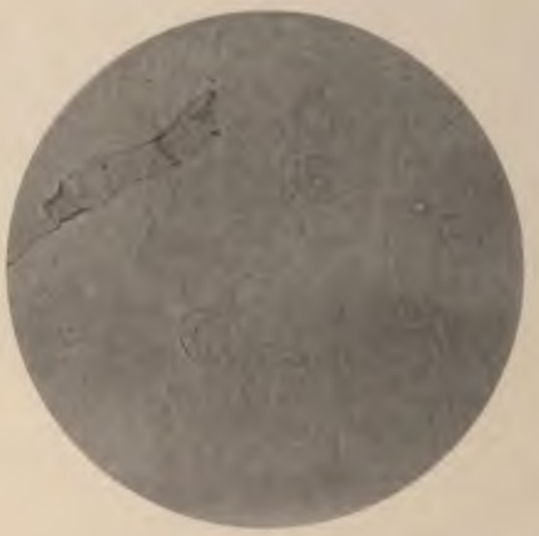
2.

3.



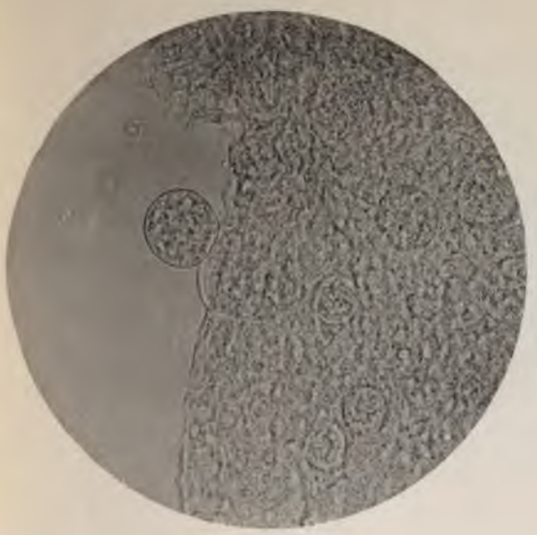
4.

5.

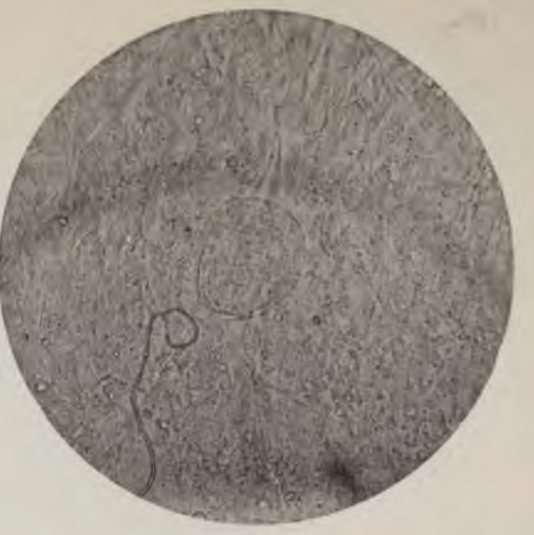


6.

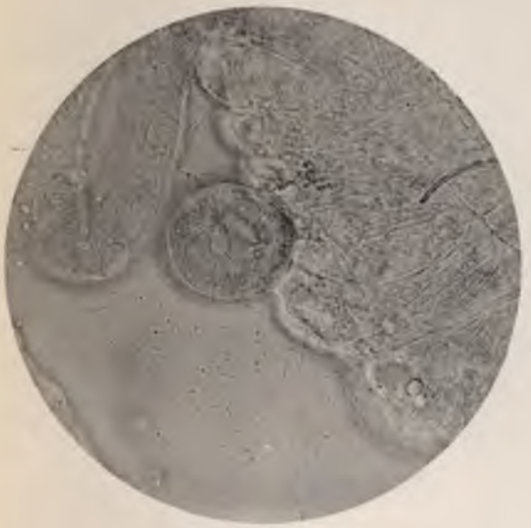
PLATE II.



7.

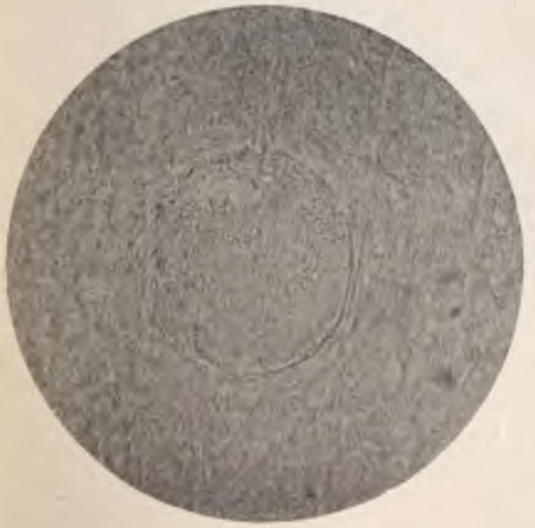


8.

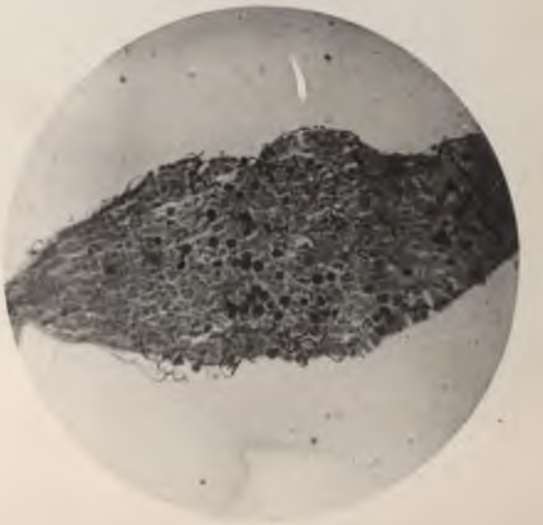


9.

10.

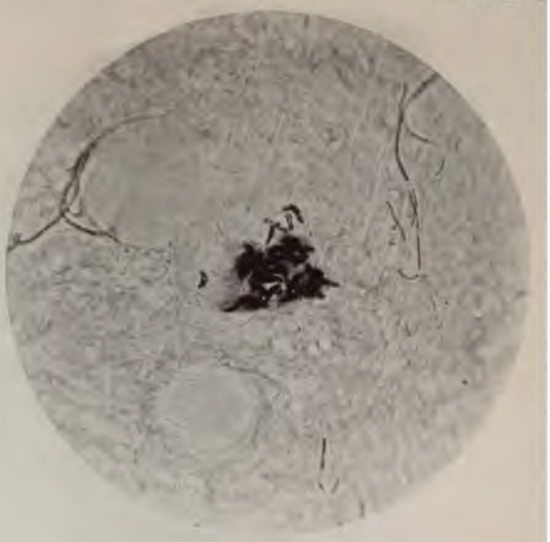


11.

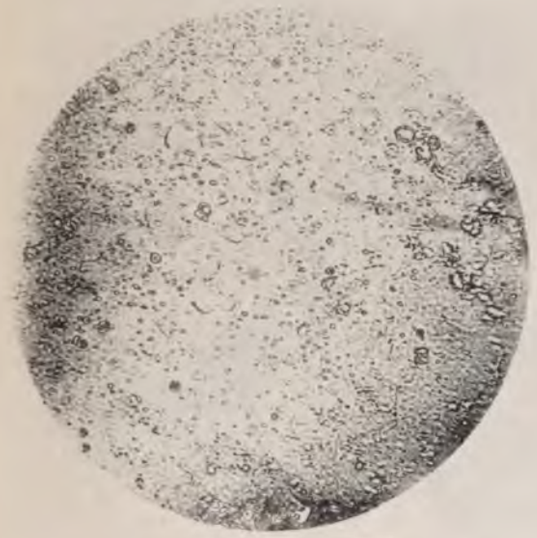


12.

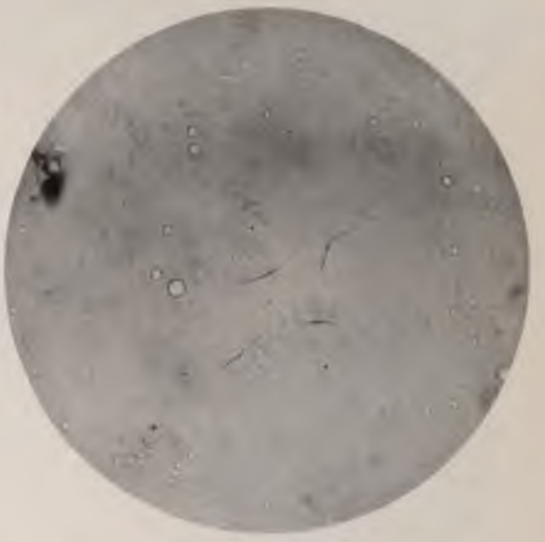
13.



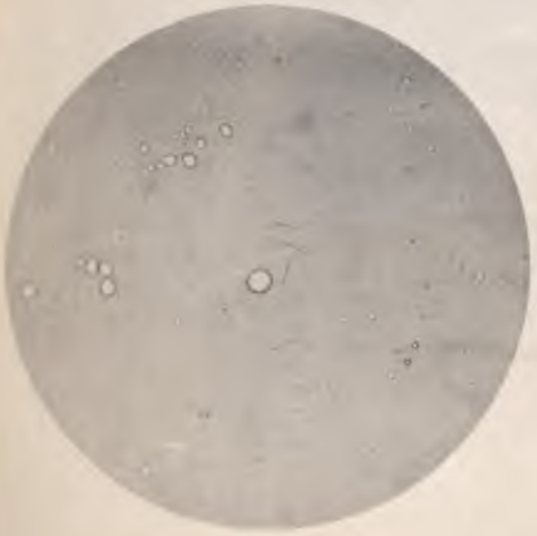
14.



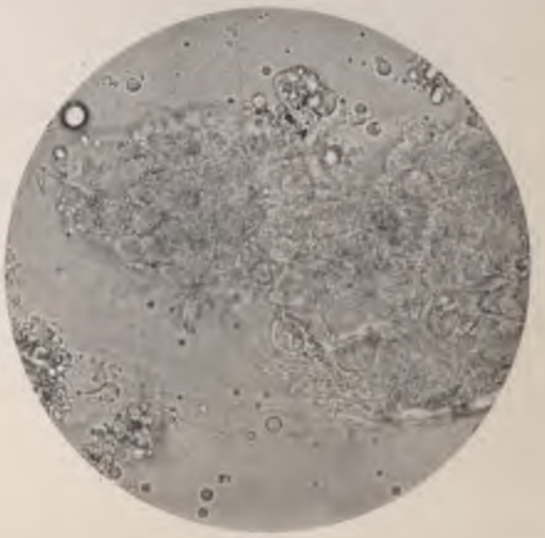
15.



16.



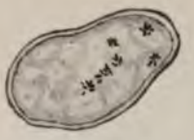
17.



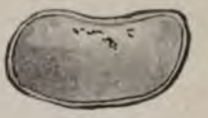
18.

PLATE IV.

1.



2.



3.

4.

5.

6.

7.

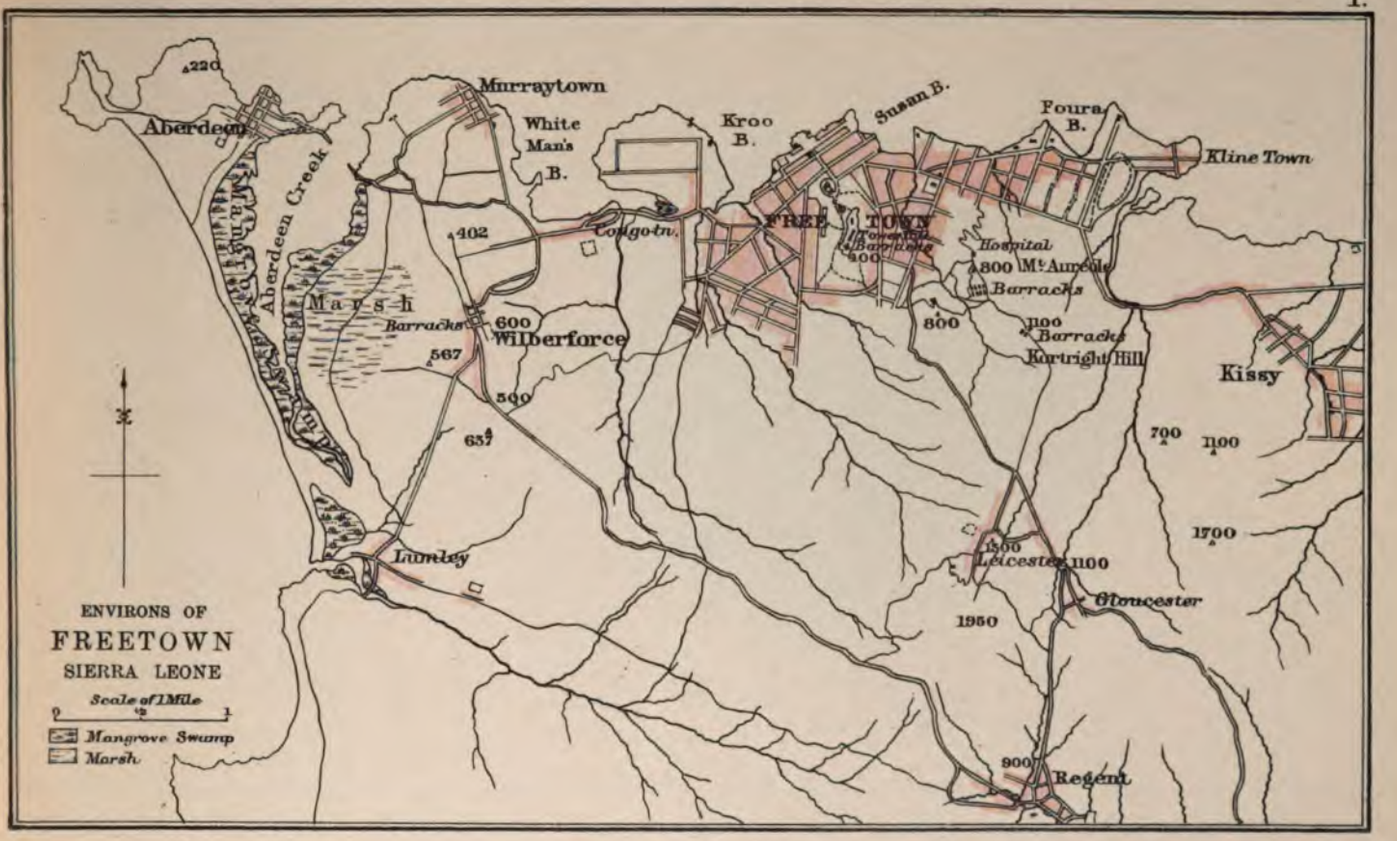
8.

9.

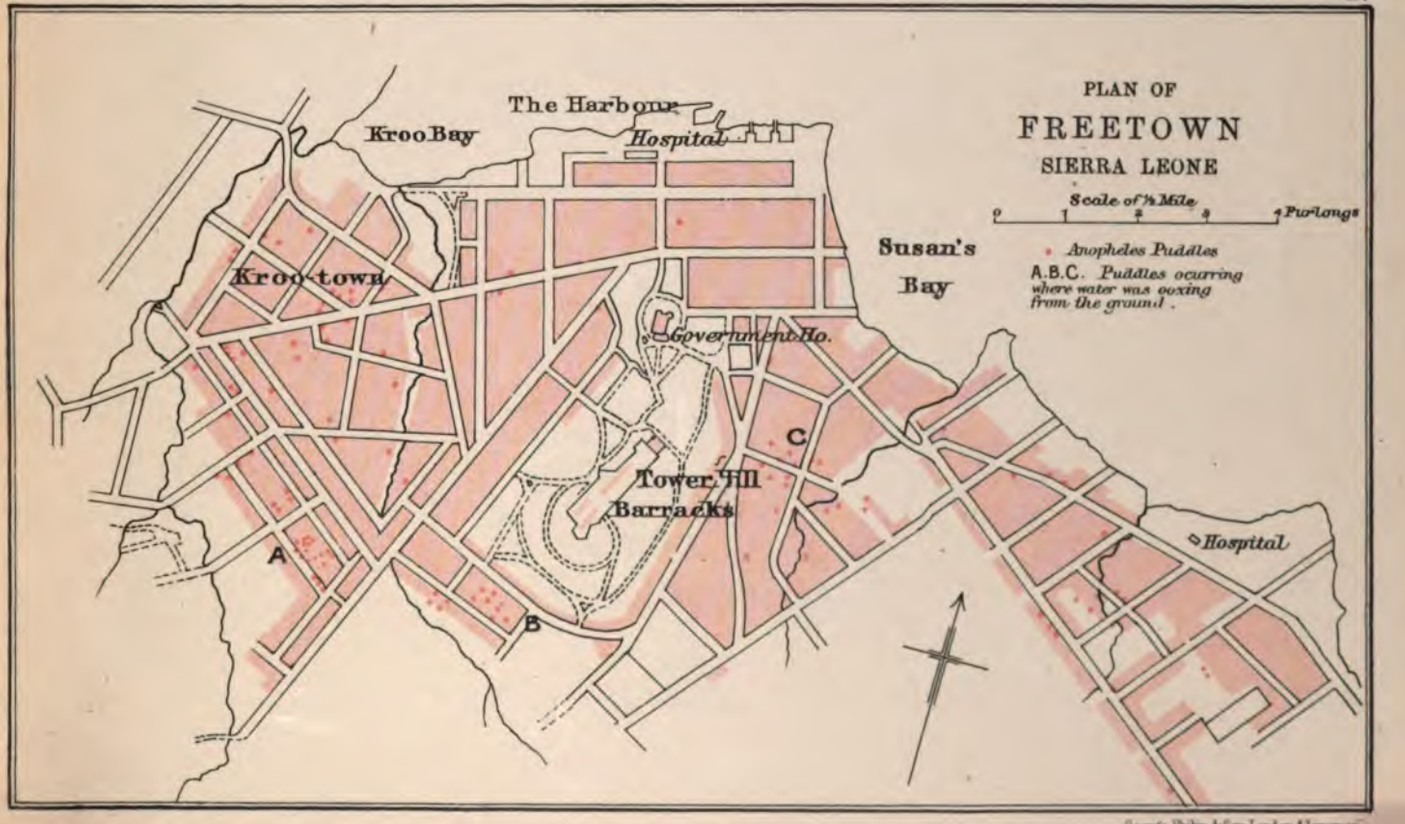
10.

11.

1.



2.



George Philip & Son, London & Liverpool

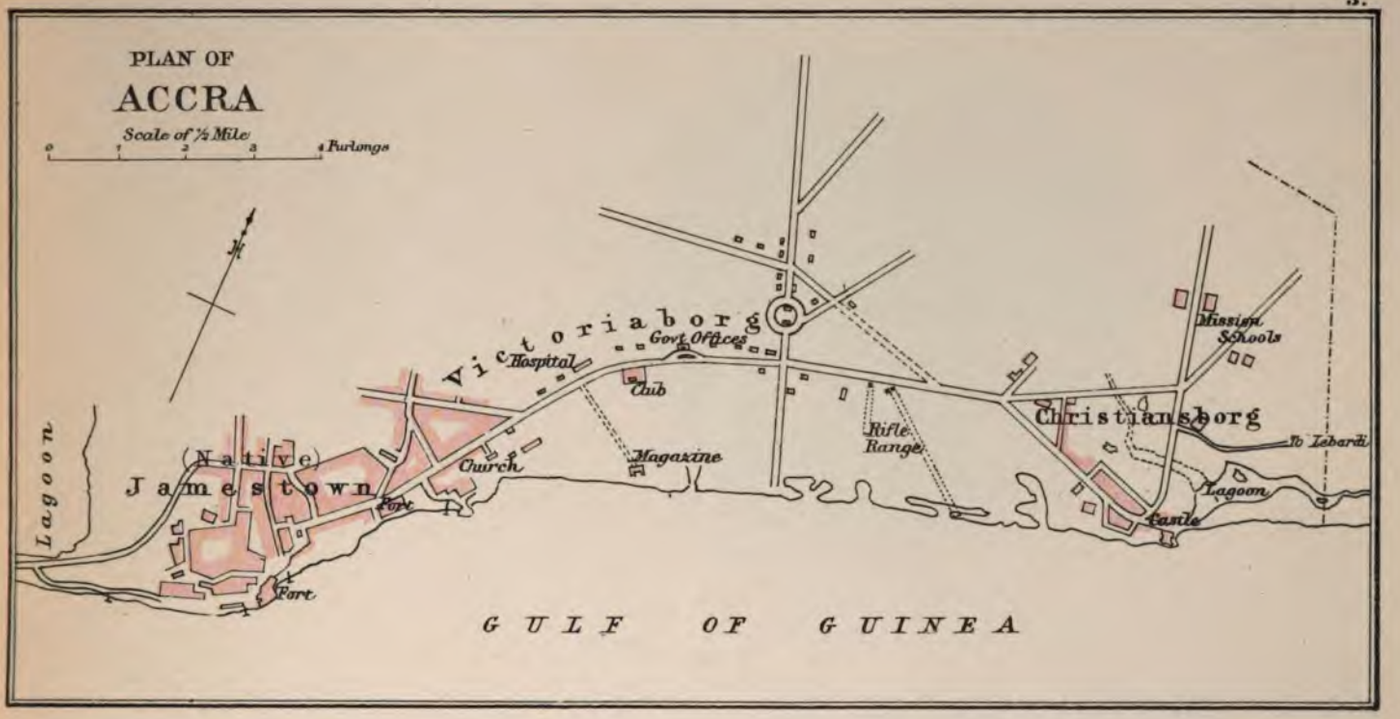
3.

PLAN OF

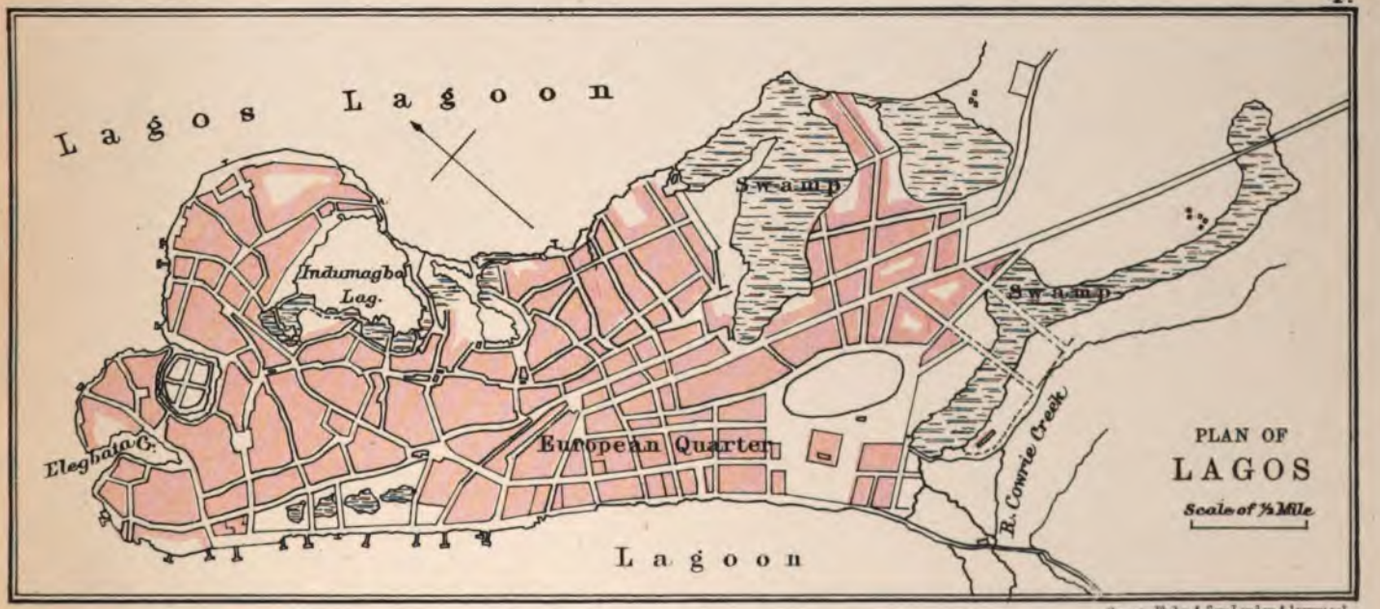
ACCRA

Scale of ½ Mile

0 1 2 3 4 Furlongs



4.



PLAN OF

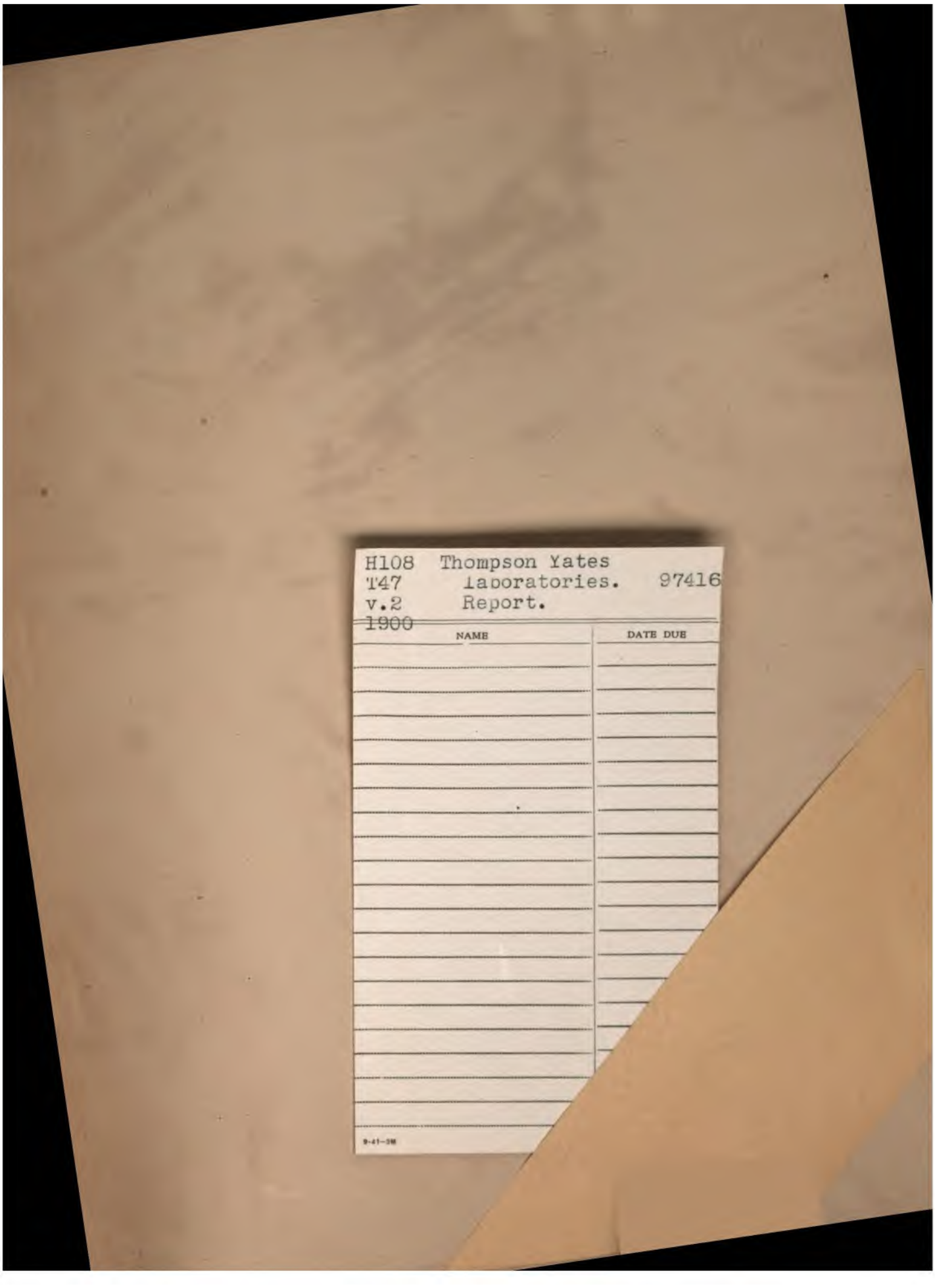
LAGOS

Scale of ½ Mile

George Philip & Son, London & Liverpool

LANE MEDICAL LIBRARY

This book should be returned on or before
the date last stamped below.


H108 Thompson Yates
T47 Laboratories. 97416
v.2 Report.
1900

NAME | DATE DUE

9-41-3M